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The life history and cytology of Protosiphon botryoides

HAROLD C. BOLD

(WITH PLATES 10–19 AND SEVEN TEXT FIGURES)

INTRODUCTION

From the numerous studies of nuclear and cell division in many types of organisms of both the plant and animal kingdoms there have emerged the striking facts of the essential similarity of karyokinesis throughout a wide range of organisms, and secondly of the apparent existence of several distinct categories of cytokinesis. In the plant kingdom especially the occurrence of varied methods of cell division has been repeatedly demonstrated. Furthermore nuclear and cell division may be closely related through the medium of the kinoplasmic achromatic figure as in the higher plants, or totally unrelated processes as in certain fungi. Reported observations of nuclear and cell division in fungi are numerous, but there have been few modern accounts dealing with the same processes in the algae and it is still a question whether the type of cytokinesis (progressive cleavage) characteristic of the coenocytic sporangia of the fungi, has a counterpart in the swarmspore and gamete formation in algal zoosporangia. McAllister's recent (1931) report of cell division by phragmoplast and cell plate in *Spirogyra setiformis* suggests that similar studies of other algae may be fruitful in clarifying the categories of cell division.

Among the various forms of Chlorophyceae whose life histories and cytological characters are still somewhat obscure are *Botrydium* Wallroth of the Heterokontae and *Protosiphon* Klebs, which up to the studies of Klebs (1896) were considered to be identical. The life history, habit of growth and cell structure of *Protosiphon* present many features which suggest simultaneously the characters of the more primitive Chlorococcales such as *Chlorococcum* and the more highly developed Siphonales. *Protosiphon* is included in the Chlorococcales by such authorities as West and Fritsch (1927), Printz (1927), Brunnthaler (1915) and Oltmanns (1922). On the other hand, Setchell and Gardner (1920) and West (1916) include the family Protosiphonaceae among the Siphonales.

The present study of these genera was undertaken primarily with the view of giving a more complete account of their life history, cell structure and cytology. The following paper deals exclusively with *Protosiphon botryoides* Klebs. This investigation was carried on in the Botanical Labo-

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ratory of Columbia University with the aid of a University Fellowship, and I take this opportunity of acknowledging my appreciation of the fellowship and of expressing my gratitude to Professors R. A. Harper and J. S. Karling for stimulating criticisms and suggestions. I should also like to thank Professors T. E. Hazen and C. C. Curtis for suggestions and valuable discussion during the progress of the work.

SUMMARY OF THE LITERATURE

The general features of the structure and reproduction of *Protosiphon botryoides* have become fairly well known through the studies of Cienkowski (1855), Rostafinski and Woronin (1877), and especially Klebs (1896). Briefly summarizing the results of these investigators, one concludes that development and reproduction of this organism may occur in several ways. First, under conditions of drought the thallus protoplast may break up into a number of more or less spherical segments or "spores" which are enclosed by walls, develop a red pigment and enter upon a resting period. Depending on subsequent conditions of their environment these "spores" may produce zoospores or grow directly into thalli. Secondly, the thalli produce zoospores when submerged in water. The zoospores may develop without fusion directly into new plants, or, conjugating in pairs, form star-shaped zygospores which after a period of dormancy germinate into new thalli. Klebs has reported the division of young cells and thalli by bipartition. Strasburger (1897) confirms Klebs' observations on the life history of *Protosiphon*. With the exception of Miss Carter's note (1926) that this alga is monoecious, there have been no other contributions to our knowledge of *Protosiphon*.

In my study of this organism I have given particular attention to the method of cytokinesis and shall include the summary of the data on this subject at this point.

Since the time of Timberlake's description of zoosporogenesis in *Hydrodictyon* there has been comparatively little detailed cytological study of the process of cytokinesis in the Chlorophyceae in general. This paucity of data is quite significant and striking in view of the fact that these algae are to many polyphyleticists the probable ancestors of many of the lower fungi and Phycomycetes, in which cytokinesis is fairly well known. In phylogenetic studies of the Chytridiales, Zygomycetes and Oomycetes considerable emphasis has been placed on characters such as the capacity to form motile reproductive cells at some time during the life cycle, as being in some manner indicative of phylogenetic relationship; but comparatively little significance has been attached to the similarity or difference in the process by which such motile cells are formed. As early as

1902 Timberlake suggested that such comparative studies of spore formation might be fruitful, but apparently few investigators have up to the present time interested themselves in this problem.

The literature of spore formation in the Thallophyta is too extensive to permit of complete review at this time. Harper (1899, 1900, 1914), Timberlake (1902) and Schwarze (1922) have adequately summarized the earlier data with especial reference to the fungi, and I shall accordingly limit my résumé to cytokinesis in the Chlorophyceae and those fungi whose spore formation is analogous to the process in *Protosiphon*.

The conception of free cell-formation which for so many decades influenced students of cell multiplication has played an important part in the observation and description of spore formation in the Thallophyta. It has been carried over in more modern times in the accounts of simultaneous cleavage by such investigators as Kusano (1909), Curtis (1921), Bristol (1920) and others. Harper (1899) and Schwarze (1922) have pointed out that the difficulties of studying thick, opaque sporangia in the living condition and at high magnification undoubtedly explain the discrepancies between the earlier accounts of spore formation and those based on more modern methods of fixation, sectioning and staining. Harper demonstrated by means of this technique that a great many stages of cleavage had been overlooked. According to his account, in *Synchytrium decipiens*, *S. Taraxaci*, *Pilobolus crystallinus* and *Sporodinia grandis*, spore formation does not consist of a simultaneous fragmentation whereby the protoplasm of the sporangium is at once cut up into a number of polyhedral segments, as Dangeard (1890) had described it in *Synchytrium;* it is rather a progressive process occupying various periods of time from about one hour in *Sporodinia* to six in *Pilobolus*. The process is markedly progressive, since furrows originate at different regions of the surface (not always simultaneously) and cut progressively deeper into the protoplasm, thus delimiting at first large segments with many nuclei, which are subsequently divided by secondary furrows into smaller, either multinucleate spores (*Sporodinia*) or uninucleate spores (protospores) (*Synchytrium decipiens*). The furrows may be quite sharp and deep as they cut through the spore plasm, or quite broad; from Rothert's (1892), Davis' (1903) and Schwarze's (1922) accounts of oogenesis and swarmspore formation in the Saprolegniales it seems to be true that the depth and width of the furrows are correlated with the volume of protoplasm within the sporangium and oogonium; broad and shallow furrows are characteristically formed when the primordial utricle is thin, while sharp and deep furrows arise when the dense protoplasm fills the cell cavity. The various genera studied show some interesting modifications of this process which are significant in the

light of the process in algal forms. In *Synchytrium decipiens* the uninucleate segments are not the definitive spores, but become multinucleate, increase tremendously in size and at germination produce the swarmspores. This period of nuclear division and cell enlargement following cleavage Harper called an embryonic stage; and the primary uninucleate segments he termed protospores. In *S. Taraxaci* the process is simplified by the omission of the protospore stage, the multinucleate segments delimited by the primary cleavage furrows functioning directly as spores which germinate as zoosporangia. In *Pilobolus* the protospores undergo a period of nuclear and cell division by constriction following which bi-nucleate spores are finally formed. In *Sporodinia* the primary cleavage furrows delimit multinucleate masses of spore plasm which round off and function as the definitive spores. The process here is essentially the same as in the other genera, but more rapid and abbreviated and produces thinner-walled spores. Harper suggested that the difference in time and conditions of germination between the spores of *Sporodinia* and *Pilobolus* may be correlated with the difference in the rates of the cleavage processes by which they are formed.

Not all investigators are in agreement as to the method of spore formation in *Synchytrium*. Kusano (1909) reports that *S. Puerariae* "does not form protospores, as is the case in *S. decipiens* (Harper, 1899, p. 488)" and has two different methods of division. The first is very similar to that described by Harper for *S. decipiens*, spore formation being accomplished by surface furrows, which cut progressively deeper into the protoplasm and with the aid of secondary furrows eventually cut out multinucleate segments. Kusano agrees with Harper that cleavage is accompanied by loss of water and extrusion of oil droplets. In addition he reports a second type of cleavage which is not accompanied by loss of water but consists of a simultaneous precipitation of membranes between the nuclei throughout the thallus, resulting in the formation of equal-sized, polyhedral segments. In view of Harper's observation that when the protospores of *S. decipiens* are completely delimited they undergo a period of rapid swelling, completely fill the sporangial cavity, and assume a polyhedral shape so that they appear to be separated by fine granular plates, one is inclined to question Kusano's description. The stage of simultaneous precipitation of membranes between the nuclei in spore formation may well be that of the swollen and closely appressed, yet completely delimited segments (embryonically germinating protospores) described by Harper. Kusano points out a supposed analogy between his process of internuclear membrane formation and Timberlake's (1902) description of zoosporogenesis in *Hydrodictyon:* "The compact cytoplasmic mass produces partitions between two adjacent nuclei, as Timberlake (1901) observed in the formation

of the swarmspores in *Hydrodictyon*." That there is no similarity between Kusano's simultaneous cleavage in *Synchytrium* and the process in *Hydrodictyon* becomes at once apparent on recalling Timberlake's description in which he states: "Cleavage itself is, as Klebs pointed out, a progressive process; . . . In the first stages in the process short furrows that have no apparent orientation with reference to one another or to the nuclei appear here and there through the cytoplasm (Fig. 22). Seen in a surface section these furrows appear as single lines and thus might easily be taken for cell plates formed in the protoplasm without the help of visible spindle fibers; but if a vertical section is studied the appearance of furrows becomes manifest (Figs. 31, 32)". "We have such coenocytic cells as the fungus sporanges and the *Hydrodictyon* cell where there is a progressive cleavage by means of surface constrictions." There exists no similarity between Kusano's simultaneous cleavage by membranes in *Synchytrium Puerariae* and the progressive cleavage in *Hydrodictyon* if we accept Timberlake's account. Neither Harper (1899) nor Kusano describes the segmentation of the spores as sporangia with the formation of swarmspores. In a more recent report on the life history and cytology of *S. fulgens*, Kusano (1930) describes formation of the spores from the thallus as a process of simultaneous cleavage by formation of "plasmic walls" between the nuclei, which is apparently similar to the process he reports in *S. Puerariae*. Another recent investigator, Curtis (1921), reports that there are about thirty-two nuclei in the thallus of *Synchytrium endobioticum* at the time of cleavage and that "the walls appear simultaneously throughout the organism" forming the spores. The spores germinate as sporangia and become highly vacuolate by absorbing water prior to zoospore formation. Miss Curtis describes the delimitation of the zoospores as follows: "Separating the vacuolated areas, and intersecting one another roughly at right angles, are short strands of protoplasm which have retained their original density, and by the combined intersections of which the delimitation of the zoospore areas is effected (fig. 63)." In the light of Swingle's (1903) observations of cleavage in *Phycomyces* sporangia, it would seem possible that the process of zoospore formation in *Synchytrium endobioticum* also can be interpreted as being accomplished through the active agency of vacuoles, rather than through the agency of the intersecting protoplasmic strands, as Miss Curtis regards it.

Kusano (1921) further finds no evidence of progressive cleavage in *Olpidium Viciae* but reports "that a clear space appeared in the cytoplasm, all at once between each two nuclei, and that the protoplasm was cut up into as many polygonal segments as there were nuclei." Wager (1913) reports that cleavage in *Polyphagus Euglenae* begins at the center

and proceeds toward the surface of the sporangium, a process very similar to that described in oogenesis and sporogenesis in *Saprolegnia*.

With the possible exception of the Saprolegniales, the Zygomycetes have been the objects of most intensive investigation as to their method of spore formation. Harper (1899), Swingle (1903) and Schwarze (1922) have studied a series of representative forms including *Pilobolus*, *Sporodinia*, *Rhizopus*, *Phycomyces*, *Mucor* and *Circinella*. These investigators agree that spore formation is accomplished by progressive cleavage by surface furrows which cut in from the periphery of the sporangium and in some cases also from the columella region. In *Phycomyces* the spores are delimited by vacuoles which gradually become angular and fuse. These authors agree that the columella is formed from a dome shaped layer of vacuoles, which become angular and fuse at their edges, thereby separating the spore plasm from the columella plasm, and in this cleft the columella wall is subsequently deposited. In *Pilobolus* in addition to the dome-shaped layer of vacuoles, cleavage furrows cutting up from the base of the sporange are active in columella formation.

The Saprolegniales show a similar process of cleavage in the formation of zoospores. Rothert (1892), Davis (1903) and Schwarze (1922) describe the formation of cleavage furrows from the surface of the central vacuole which cut centrifugally through the protoplasm, isolating the zoospore initials. As the cleavage furrows cut through the plasma membrane contraction occurs due to the escape of cell sap through the sporangium wall. The delimited spore initials round up and then expand, forming closely appressed polygonal masses, between which the cleavage furrows appear as delicate lines. Then follows a second contraction stage during which the spores separate, round up and become mature. Schwarze points out that the rapidity with which the spore initials are delimited, contract, and expand, and the fact that there are two contraction stages have led to the false conclusion that spore formation is simultaneous. In addition to sporangia in which the protoplasm occurs as a peripheral layer surrounding a large central vacuole, there are others densely filled throughout. In the first type Rothert describes the heaping up of the protoplasm at definite regions along the plasma membrane which thus become separated by broad furrow-like areas. According to Schwarze the furrows deepen progressively in cutting through the spore plasm, but Rothert maintained that they appeared simultaneously in their entire depth. Cleavage in densely filled sporangia without a central vacuole is effected through the formation of a central, longitudinal cleft, from which smaller furrows cut through the spore plasm in centrifugal fashion toward the surface.

In the Myxomycetes as well, the evidence in the literature points

strongly to spore formation by progressive cleavage. In *Ceratiomyxa* of the Exosporeae Olive (1907) found that the fruiting stalks were composed of a central layer of gelatinous material on whose surface is spread a very thin layer of multinucleate spore-plasm which is later divided by broad furrows into uninucleate segments. Olive notes that the surface spore-plasm of one region of the stalk may have reached the condition of uninucleate segments while in other regions cleavage is just beginning. No nuclear divisions occur immediately before or during cleavage. The uninucleate segments or protospores round off and become the definitive spores, borne on sterigmata. Olive describes the spore nuclei as passing through a period of synapsis followed by two rapid nuclear divisions so that the mature spores are tetranucleate. He regards these two nuclear divisions as reduction divisions. In general they would correspond to the embryonic stage which Harper described.

Of the Endosporeae Harper (1900, 1914) has studied *Fuligo* and *Didymium*. In *Fuligo* the nuclei are more or less aggregated into groups before cleavage begins. Furrows form at the surface of the aethalium and cut in at all angles. They may be narrow with sharp edges, or broad, curved and forked. The primary cleavage furrows do not cut through the entire mass of spore-plasm but curve and fork so as to delimit superficial segments. New furrows not continuous with the first appear and cut up the central protoplasm into multinucleate blocks. Meanwhile the surface segments are further subdivided into fragments with one or several nuclei. The central portion of the aethalium, however, may be still multinucleate and undivided by the time the surface segments have reached the uninucleate stage. At the time the superficial segments are forming, nuclear divisions begin and continue during the entire process without any apparent direct relation to it. The ultimate cleavage segments function as spores without further divisions and growth such as occur in *Synchytrium decipiens*.

Cleavage in *Didymium* is essentially similar to that described for *Fuligo*. Radial furrows appear along the capillitial threads near the columella and on the outer surface of the sporangium. Later tangential furrows originate at the primary furrows, and move into the protoplasm, delimiting small multinucleate masses. These are further subdivided until the uninucleate segments are formed. These function directly as the definitive spores as in *Fuligo*. Probably only a single division of the nuclei takes place while cleavage transpires. Bisby (1914) described a similar method of cleavage in the sporangia of *Physarella mirabilis* and *Stemonitis fusca*. Vonwiller (1919) figures spore formation in *Lycogala* as a process of progressive cleavage proceeding from the center toward the periphery of the maturing fruiting body. More recently Howard (1931) in a detailed account of the

life history of *Physarum polycephalum* describes and figures spore formation as progressive cleavage by furrowing, very similar to that which Harper had described in *Fuligo*.

It would appear from the above mentioned cases that the bulk of the evidence indicates that spore formation in the coenocytic sporangia of Chytrids, Zygomycetes, Oomycetes and Myxomycetes is by progressive cleavage.

As to the method of cytokinesis in the Chlorophyceae fewer data are available. This is doubtless to be accounted for in part by the presence of chlorophyll, starch grains, and oil droplets, which makes the study of spore formation more difficult than in the fungi. Many of the earlier students of the algae regarded spore formation as a simultaneous division of the protoplasm in the zoosporangium. Such an account is given by Famintzin (1871) for *Chlorococcum infusionum*. Cohn's (1875) description of spore formation in his newly described *Chlorochytrium Lemnae* suggests that the process is somewhat similar to that in *Synchytrium decipiens*, and in certain respects to that in the densely filled sporangia of *Saprolegnia*. Cohn describes this process as follows: "Endlich tritt in dem grünen Protoplasma eine eigenthümliche Art der freien Zellbildung auf, indem sich an verschiedenen Punkten der Zellhöhle in der Nähe der Wand Ansammlungen des grünen Inhalts bilden, die nach innen vorspringende Wellenberge darstellen, und durch Wellenthälern von geringerer Tiefe unter einander getrennt sind (Fig. 5g). Indem das in den Wellenthälern enthaltene grüne Plasma allmählich ganz und gar nach den Wellenbergen wandert werden diese von einander völlig isolirt; so zerfällt das grüne Plasma der Endophytenzelle in eine grosse Zahl von Segmenten, die, gleich Dotterkugeln eines gefurchten Froschei, dicht an einander gelagert sind (Fig. 5, e, f.). . . . Schliesslich zerfallen die segmente wieder in einer Weise, die ich wegen ihrer Undurchsichtigkeit nicht specieller zu verfolgen vermochte (Fig. 5 g. h.), in einer ausserordentlich grosse Zahl birnformiger Zoosporen, welche dicht aneinandergedrängt, die Höhle ihrer Mutterzelle ausfüllen (Fig. 5 g. h.)." The similarity between the polygonal segments which Cohn describes and figures and the uninucleate protospores of *Synchytrium decipiens* is very striking, and the further development of these segments into swarmspores in *Chlorochytrium* seems comparable to the embryonic stage in *Synchytrium*. Klebs (1881) describes zoosporogenesis in *C. Lemnae* as a process of successive bi-partition. Miss Bristol (1920) does not comment on sporogenesis in *C. Lemnae* Cohn, but reports in *C. bienne* that the process is one of bi-partition. Furthermore, in *C. paradoxum* she reports that the protoplast contracts into an irregular globose mass, and the space between its boundary and the cell wall becomes filled with

reddish or orange colored granules. This mass divides into two parts, each of which by further bipartition forms zoospores. The author adds: "During this process of division a rejuvenation of the protoplasm takes place, so that the completed zoospores entirely fill the cell cavity." Miss Bristol does not figure this process of bipartition, but several points in her discussion indicate strongly that the process may be progressive cleavage. The loss of water and contraction of the protoplast before or during the spore-forming divisions, the elimination of waste and granular material from the protoplast, and the so called "rejuvenation"—swelling of the spore initials, are phenomena usually associated with the progressive cleavage of a coenocyte. Miss Bristol (1917) described a new species of *Chlorochytrium, C. grande,* which produces both zoospores and aplanospores which are morphologically equivalent; however, she states that the former arise by bipartition of the protoplast and the latter by its simultaneous division.

In a more recent paper Geitler (1924) attempts to summarize the data on spore formation in the unicellular forms (Protococcales) in connection with his study of the development and structure of *Sorastrum spinulosum.* At maturity the cells of this organism are multinucleate and during zoospore formation the chromatophore divides by successive bipartition. According to him: "Die Fortpflanzung beginnt mit Teilungen des Chromatophors, der in mehrere Stücke, soviel als später Zoosporen enstehen, zerlegt wird. Meist teilt sich der Chromatophor zuerst in zwei Hälften, die dann Sukzessive in weitere Stücke zerlegt werden." The protoplast is divided by one or several clefts which may appear simultaneously; he states that part of the protoplasm does not enter into the zoospores but remains in the mother cell. He stresses the similarity between the process in *Sorastrum* and that in *Pediastrum, Tetraedron, Characium* and *Hydrodictyon* and concludes: "Dass Characteristische für alle diese Formen ist, dass die Tochterzellen simultan, durch Zerklüftung des vielkernigen Plasmas enstehen, wobei das ungeteilte Pyrenoid aufgelöst und in den Tochterzellen neu gebildet wird." In view of Geitler's own description of zoospore formation in *Sorastrum,* Smith's (1916) and Harper's (1918b) accounts of *Pediastrum,* Smith's account of *Characium* (1916), *Tetraedron* (1918), *Scenedesmus* (1914) and Timberlake's account of *Hydrodictyon* (1902), it is difficult to understand why Geitler should state that these genera show simultaneous cleavage. On this same basis Geitler would divide the Protococcales into two groups. In the first class he would include forms such as "*Tetraedron, Characium, Scenedesmus, Coelastrum, Sorastrum, Pediastrum, Hydrodictyon*" which have "simultan" formation of the reproductive cells, while the second group would include genera like "*Chlorococcum, Chloro-*

chytrium, Cystococcus, Chlorella, Dictyosphaerium" in which the daughter cells are formed by successive bipartition. While one must commend Geitler's recognition of the fact that the finer details of cell structure and multiplication must be considered in phylogenetic systems, one can hardly agree with his present classification. His inclusion of *Chlorochytrium* and *Chlorococcum* in his second group is open to criticism. I have pointed out above the evidence that zoosporogenesis in *Chlorochytrium* is not by bipartition. There is some diversity of opinion as to zoospore formation in *Chlorococcum*. Famintzin (1871) reports that it is a simultaneous process in *C. infusionum*, while Artari (1892) describes it as bipartition in the same species. In my study of the same species (1931) I found that the swarmspores arose in the mother cell by a progressive cleavage of the protoplast in a manner very similar to that in *Sporodinia*. The aplanospores of *Chlorococcum* which are simply zoospores which, due to lack of moisture, are not liberated and omit the motile stage, are formed in an identical manner. In her account of *C. humicola* (1919) Bristol also emphasizes the complete homology between zoospore and aplanospore, and yet reports that they have a very different method of formation, the former by repeated bipartition and the latter by simultaneous fragmentation of the protoplasm of the parent cell. No explanation of this apparent contradiction is vouchsafed.

The remaining genera discussed by Geitler as far as I am aware have not been studied with particular attention to cell division. Among the Protococcales therefore two types of cell structure and division occur: the first category includes forms in which the cells are uninucleate and the daughter cells formed by bipartition, such as *Scenedesmus, Tetradesmus*, and probably *Chlorella;* the second would include such genera as *Chlorococcum* (certainly *C. infusionum*), *Hydrodictyon, Characium* and probably *Sorastrum* and *Chlorochytrium*, forms in which the cells are coenocytic at least immediately preceding reproduction, and in which the reproductive cells are formed by progressive cleavage of the protoplast. I have omitted *Pediastrum* from both groups in view of the contradictory evidence available.

Of the Volvocales the conditions in *Chlorogonium, Gonium* and *Eudorina* may be considered typical of the group. Hartmann (1919) describes zoospore formation in the first genus as effected by a series of successive nuclear and protoplast bipartitions so that the number of spores produced is usually a multiple of two. A similar process with gelatinization of the mother cell wall is involved in the production of the daughter colonies in *Gonium* (Harper, 1912, Hartmann 1924) and in *Eudorina* (Hartmann, 1924).

Those members of the Ulotrichales which have been studied seem to indicate that the process here is similar to that in the Volvocales. Strasburger (1880), Gross (1931) and Cholnoky (1932) report that the zoospores and gametes of several *Ulothrix* species arise by consecutive nuclear divisions and bipartitions of the protoplast. Cholnoky (1928) describes a similar process in *Stigeoclonium tenue.*

There are several accounts of spore formation in *Cladophora.* Strasburger (1892) states that the zoospores in *C. laetevirens* and *C. lepidula* arise by clefts in the mother cell: "Zugleich wird der Wandbeleg durch rinnenformige, von innen nach aussen vordringende Vertiefungen in so viele Abschnitte zerlegt, als Zellkerne vorhanden sind." The primordial utricle is divided into polygonal masses by delicate plasma walls; according to Strasburger nuclear division precedes cleavage; the pyrenoids dissolve and arise *de novo* in the swarmspores, which contract toward the center of the cell when cleavage is completed as in *Hydrodictyon;* cilia develop from a kinoplasmic swelling of the plasma membrane. He describes an essentially similar process in *Bryopsis, Acetabularia, Siphonocladus,* and *Chaetomorpha.*

A more recent account based on fixed, sectioned and stained material is that of Miss Czempyrek (1930). In *Cladophora callicoma* and *C. glomerata* zoospore formation is initiated at the terminal cells of the branch and proceeds toward the basal; the nuclei multiply rapidly in preparation for swarmspore production; then the small chromatophore segments begin to collect around the nuclei as does the protoplasm, by a sort of 'heaping up' or flowing process. This description reminds one of Rothert's (1892) account of spore formation in those sporangia of *Saprolegnia* with central vacuoles. The threads of protoplasm still connecting the swarmspore initials are described as becoming finer until they ultimately disappear, leaving the spores completely delimited. There is some relation between this localized accumulation of protoplasm in *Cladophora* and Swingle's theory of localized contraction. In *C. glomerata* the chromatophore is more homogeneous. The protoplast contracts somewhat, the nuclei assume a peripheral position and the zoospore initials "kugeln sich entweder einzeln direkt vom übrigen noch unzerteilten Plasmamantel ab" etc. Although the author does not interpret the process in *C. glomerata* as one of progressive cleavage, her figures are very similar to Swingle's figures of *Rhizopus,* with the exception that uninucleate segments are formed immediately in *Cladophora.* Klebahn (1899) describes and figures oogenesis in *Sphaeroplea annulina* as effected by surface furrows which progressively cut through the protoplasm, delimiting the egg initials.

Davis (1908) reports the development of the large multiciliate zoospores

of *Derbesia* as a process of progressive cleavage by furrows which originate at the surface of the sporangium, delimiting first multinucleate and ultimately uninucleate segments. Miss Williams' (1925) account of the development of the gametangia in *Codium tomentosum* does not particularly discuss the method of formation of the male and female gametes from the multinucleate gametangia, but she suggests that the gametes are delimited simultaneously by walls.

MATERIAL AND METHODS

My original *Protosiphon* material was collected from clay soil from several different localities in the vicinity of New York City and was found intermingled with *Botrydium* on the same substratum. The soil which adhered to the plants made fixation and sectioning difficult and stimulated an effort to obtain pure cultures on artificial media. This was accomplished in the following manner. As the soil cultures of the plants slowly dried in bright sunlight the protoplasm of the thalli broke up into a number of rounded cells which Cienkowski (1855) first called 'spores.' With the help of a binocular dissecting microscope and finely pointed glass needles, groups of these socalled spores (cysts) or single thalli with their enclosed cysts were readily picked from the soil, rinsed several times in sterile water, and transferred singly to drops of water on the surface of nutrient agar in a petri dish. Several hours afterwards numerous zoospores were produced which were then transferred to further nutrient media. Repeated transfers in this manner, of the progeny of single thalli thus gave uni-algal cultures within several days. All fungi were eradicated from the cultures by these repeated transfers and growth of bacteria was very slow and negligible in effect. Detmer's nutrient medium in concentration of 1.75 grams per 1000, solidified with agar to a consistency of 2%, was used exclusively. Several separate isolations of the organism from different localities were made by the methods described above to determine whether or not there were any differences. For comparison cultures of *Protosiphon botryoides* were also secured from the Pflanzenphysiologisches Institut der deutschen Universität in Prague (Pringsheim 1929), and it was found that all forms isolated from different localities were identical with the European one. Cultures were grown on agar slants, in petri dishes and erlenmeyer flasks in a moist atmosphere and protected from direct sunlight.

For cytological studies the method employed by Schwarze (1922) in his study of the water molds was used. Material for fixation was grown on agar in petri dishes. Drops of fixative were placed on the plants with a pipette and subsequently, melted agar near the point of solidification was poured over the plants. The agar solidified almost immediately and small

blocks containing the algae were cut out and dropped into vials containing a large quantity of fixative. These agar blocks with the thalli were washed, dehydrated, imbedded and sectioned in the usual way. In this manner the plants which at certain stages do not adhere firmly to the substratum are prevented from floating off during washing and dehydration. Of a variety of fixatives Flemming's weaker solution and Allen and Wilson's modification of Bouin's fluid (Allen's B-15) produced the most satisfactory preparations. Sections were stained in Flemming's triple stain or in Haidenhain's iron haematoxylin. The former in general was more differential. A modification of the usual technique was employed with the free swimming zoospores. To slides coated with a thin film of egg albumin were added drops of water containing the motile cells. These were mixed with a small drop of Flemming's weaker fluid and allowed to evaporate. When the margin of the drop was quite dry, the slides were immersed in alcohol and stained in the same manner as sections.

CULTURAL CHARACTERS

When a group of plants is placed on the surface of freshly poured and cooled Detmer's agar, numerous zoospores are produced within a few hours and swarm radially for considerable distances from the point of inoculation, forming discoidal colonies where they come to rest. If the number of swarmers is large they form dense films whose individuals are angular except at the free surfaces. They are very sensitive to light variations and the shape of the young colony is to a large extent determined by light intensity, unilateral light producing asymmetrical colonies. The extent of the water film on the surface of the substratum determines the density and radius of the colony. As the zoospores develop their apices are lifted above the surface of the medium so that the rhizoidal portion alone is in contact with the substratum. This is true only for densely inoculated cultures where the plants are mutually supported. Isolated plants have the tendency to be more procumbent. The thalli in mature and crowded cultures may extend as high as several millimeters above the agar surface.

The color of the plant mass changes with age from a bright yellow-green, through green to dark olive green, and with the combination of bright light and low moisture-content of the medium, becomes bright brick red. Cultures from two to four weeks old have a shiny, glistening appearance, which later becomes dull and powdery due to the endogenous segmentation of the transparent thalli into numbers of dense and opaque cysts.

Observations of hundreds of cultures on solid media so far indicate that the first swarming period after inoculation, which may continue for several

consecutive days, is the only one until the plants are fully grown. This is perhaps due to the decreased moisture-content of the agar resulting from evaporation. Unless the plants are later submerged either by adding water or by droplets of condensing water vapor, no further multiplication is accomplished by zoospore formation. The water of condensation is often sufficient stimulus to initiate zoospore production, but unless it submerges the plants, the zoospore anlagen do not leave the parent thallus. Instead they develop walls and subsequently become new plants. Similar correlations between culture conditions and development were found in *Chlorococcum infusionum* (Bold, 1931). In addition to these inhibited zoospores cell multiplication is accomplished by separation of buds and lobes from young thalli by septa, as shown in figures 74 and 75. Growth on solid media is confined to the surface of the substratum, and in such cultures approximately one month is required for zoospores to develop into mature thalli.

THALLUS STRUCTURE

The *Protosiphon* thallus attains its best development on very moist soil cultures. Under such conditions the aerial portion of a single plant may extend half a millimeter above the surface of the substratum with the unbranched rhizoid penetrating the soil to a depth of a millimeter. The subterranean portion is quite colorless and contains a thin layer of protoplasm surrounding one continuous vacuole, or a series of central vacuoles. Chlorophyll is developed only in the aerial, bulbous portion of the plant.

Growth and development in agar cultures is only slightly inferior to that on soil. Under crowded conditions the aerial portion of young plants may become lobed and branched, and often such branches and buds are cut off from the parent plant by definite partitions as noted above. I have been unable so far to follow this process of septation because it occurs in agar cultures and as soon as the plants are placed in drop cultures either all development ceases (if the plants are young) or zoospore formation soon occurs. When a large number of individuals are growing together on a small surface the cell shape is generally narrower, and the bulbous portion tapers more gradually into the rhizoid.

The central region of the thallus is occupied by a large vacuole which may be continuous from the base of the rhizoid to the apex of the thallus or consist of several vacuoles separated by thin laminae of cytoplasm (figs. 69–75, text fig. 2). The primordial utricle extends deeply into the cell cavity in the bulbous, expanded portion of the thallus but nearer the rhizoid it becomes thinner and sometimes membranous (figs. 2, 3). From observation of living and stained preparations I have been unable to dis-

tinguish a differentiated chlorophyll-bearing region of the cytoplasm or definite chromatophore such as occurs in the cells of *Botrydium*, *Vaucheria*, *Nitella*, and the phanerogams. The chlorophyll completely pervades the cytoplasm of the upper portion of the cell and shades off imperceptibly into the colorless cytoplasm in the rhizoidal region. Further evidence for this is available from study of sectioned material, in which no distinction be-between colorless and chlorophyll-bearing cytoplasm is apparent (figs. 1, 25, 39, 40). Pyrenoids are present both in the peripheral and deeper protoplasm, and the nuclei are more or less evenly distributed between the plasma membrane and tonoplast. According to my observations the *Protosiphon* thallus is in this respect very similar to that of *Hydrodictyon* in which Timberlake (1901) reports the absence of a specially differentiated chromatophore though Klebs (1891) described a differentiated net-like chromatophore as he did also for *Protosiphon*.

In general the pyrenoid is surrounded by a number of starch grains which are evident in the living cells and stain typically with aqueous iodine-potassium iodide solution. Starch is most abundantly developed around the pyrenoid in thalli which are entering the encysted condition (figs. 6–10, 38, 39). In favorable preparations stained with the Flemming triple stain the pyrenoid appears a brilliant ruby red, while the surrounding halo of blue starch grains is imbedded in the orange tinted cytoplasm. While the pyrenoid is sometimes homogeneous and spherical, it more often appears either vacuolate or fissured and segmented as in figures 6–10. In such segmented pyrenoids the central and continuous mass of the body is typically ruby-red in color, while the peripheral segments which split off stain more opaquely and are of purplish-red tint. The starch grains surrounding the pyrenoid take up the gentian violet with avidity. A careful study of the staining reactions of the pyrenoid mass, the segments which split off from it and the enveloping starch grains, in addition to the relative position of the starch segments with respect to the pyrenoid itself, show that the fragments or portions of the pyrenoid which are separated from it become transformed into starch grains. In optical section the starch segments are parenthetic or wedge-shaped sectors; careful focusing shows that together they often form the surface of an incomplete, hollow sphere. In many preparations the pyrenoids are imbedded in a region which stains more heavily with Orange G than the general cytoplasm (fig. 1). It may be that in these regions the insoluble starch has been made available for metabolism by transformation into another form of carbohydrate, although I have found no evidence of hydrolysis of the peripheral starch segments which adjoin this orange-staining region. The relation of these observations to those of other investigators will be discussed later.

The mature thalli are always multinucleate, this condition arising very early in the development of the zoospore (figs. 19–23). The zoospore is uninucleate when liberated from the parent cell and after coming to rest, but the nucleus soon divides, forming a progressively larger number as the thallus develops. Unlike *Synchytrium* in which the vegetative thallus is uninucleate (Harper, 1899) until immediately before reproduction, the thallus of *Protosiphon* is coenocytic throughout its vegetative development. Klebs (1896) states that the nuclei are confined to the deeper, colorless cytoplasm, but I am convinced from study of sections (fig. 1, etc.) that they are evenly distributed between the vacuole and cell wall. Although small, the nuclei have an appearance strikingly like that of the higher plants (figs. 1,11,12, 25–28). With the Flemming triple stain usually a single red nucleolus and blue chromatin material are clearly visible within the nuclear membrane. The chromatin may be largely confined to the periphery of the nucleus (fig. 11), although more often it is distributed as a reticulum through the nuclear cavity (fig. 12). The view of Golenkin (1899) and others that in many algae the nucleus is of primitive organization with chromatin aggregated in the center in the form of a large karyosome nucleolus is not tenable in *Protosiphon* as far as my observations go; for in addition to the safranin-staining nucleolus a well developed chromatin reticulum is always present and the nucleole apparently plays no rôle in the formation of the chromosomes. I have so far not found a complete series of the successive stages of nuclear division, but the large number of isolated stages observed show clearly that the process is essentially similar to that in the higher plants so far as the formation of the chromosomes is concerned.

The chromatin of the resting nucleus during early prophase becomes spireme-like (figs. 13, 14) and is then segmented into a number of chromosomes which lie scattered in the nuclear cavity. Spindle fibers develop from both poles of the nucleus and the chromosomes become arranged at the equatorial plate (fig. 15). I have so far been unable to trace the origin of the spindle figure prior to the equatorial plate stage. At this phase the division figure lies within a clear zone and the spindle is distinctly bi-polar and stains violet in the triple stain. Study of a large number of nuclear division stages has convinced me that there is typically a centrosome present at each pole of the spindle (figs. 15, 16). In a great number of preparations the cytoplasm near the poles of the spindle is somewhat densely granular, but I have found no evidence of astral radiations. A great number of division figures are so oriented with respect to the plane of section that the poles are not plainly visible, and though the spindle fibers seem to converge, the centrosome in such figures does not appear at the pole. In more

favorable preparations there is a sharply red-staining granule at the point where the fibers converge. In certain preparations a single (fig. 14) or two (fig. 13) deeply staining bodies are visible on the nuclear membrane and may represent isolated stages of the division of the centrosome. The division figure is intra-nuclear (figs. 18, 15) at least to the equatorial plate stage, but with the subsequent elongation of the spindle in anaphases and telophases it is not so sharply delimited from the cytoplasm. As it elongates the spindle may become slightly curved. The chromosomes are densely aggregated when they reach the poles and from these groups the daughter nuclei are reorganized. In my preparations the chromosomes are very minute and densely crowded at the equatorial plate stage, and for this reason it is almost impossible to make an accurate count. In the most favorable preparations the number seems to be fairly large, and approximately twelve have been counted in several figures.

Synchrony of nuclear division in coenocytes and syncytia both in plants and animals has been often reported, and Karling (1928) has summarized the more important available data. He distinguishes between complete synchronism in which all the nuclei of one cell divide simultaneously and are in the same stage of division at the same time, and progressive synchronism in which division is simultaneous but the seriation of stages discontinuous. Nuclear division in thalli of *Protosiphon* belongs to the second category (fig. 18). This figure shows that all nuclei are dividing simultaneously, but those at the apex are in the anaphase condition while the remainder are in the equatorial plate stage. The nuclear divisions in the protospores at the conclusion of cleavage more nearly approach complete synchrony (fig. 34). In the thalli, while the nuclei at the apex of the sac may be further advanced in division than those at the rhizoidal region as in figure 18, the opposite relation also obtains; so that nuclei at the rhizoidal portion may be more advanced than those at the apex. Similar observations have been reported by Strasburger (1880), Soltwedel (1882) and Nêmec (1910) for the embryo sacs of angiosperms.

The protoplasm in developing thalli and young coenocysts is characteristically highly vacuolate (figs. 1, 4, 25–27, text fig. 2). The primordial utricle may be almost completely perforated with rather large vacuoles In many cases these intra-utricular vacuoles appear to be bounded by rather definite membranes which show avidity for the violet of the triple stain (figs. 27, 4, 5). The protoplast is bounded both externally from the wall and internally from the central vacuole by rather sharply differentiated membranes which become conspicuous during cleavage (figs. 29, 32) or when slight shrinkage is caused by reagents (figs. 5, 5, a). Figure 5, a, represents a more highly magnified view of a small portion of the pri-

mordial utricle of the same cell shown in figure 5. The interior membrane or tonoplast which separates the protoplast from the vacuole has been separated from the protoplasm by the action of reagents, though some cytoplasm still adheres to it.

The cell wall in all stages with the exception of old cysts and zygotes is quite thin. It swells slightly and stains intensely blue when treated with sulphuric acid and iodine-potassium iodide solution, suggesting a cellulose composition. I have not secured any evidence of lamellation. In mature cysts maintained under conditions of drought the wall is markedly thickened.

LIFE HISTORY AND REPRODUCTION

My observations confirm those of Klebs as to the life history of *Protosiphon*. Examination of the zoospores formed after the inoculation of a new culture shows many of them fusing in pairs (fig. 67), and a large number also coming to rest without conjugation. During the first twenty-four hours both fused and single zoospores tend to round up and develop walls. The zygotes and single zoospores cannot be distinguished from each other on the basis of size alone, since there is a wide range of variation in the size of the zoospores themselves. It is thus not uncommon to find a young zygote smaller in volume than a single zoospore. The zygote during the next forty-eight hours becomes irregular in shape, its wall thickens irregularly and the contents become dense and opaque (fig. 69a). I have frequently followed the complete development of the zoospore into the mature thallus and figures 69–73 illustrate some of the phases from daily study of developing cultures. These cultures were inoculated at 9:30 A.M. and by 12 noon were rapidly producing zoospores. Figure 67, i, shows young zygotes and zoospores from the culture at the evening of the first day. The zygotes show clearly two stigmas, and the presence of two pyrenoids is in many cases indicative of fusion, while the zoospores on the other hand possess but a single stigma. Twenty-four hours later (fig. 69, a) the zygotes have become rather opaque and their wall rather irregular. In the same interval the zoospores have become either equal or larger in size than the zygospores (fig. 69), have developed walls, and many of them have begun to change their shape by enlarging somewhat at one pole. The pyrenoids are quite conspicuous with a surrounding layer of starch segments, and in some cases have divided or seem to be dividing. The central vacuole increases in size and is separated from the enlarging pole by a thin layer of protoplasm. There is no sharp distinction between chlorophyll-bearing and colorless cytoplasm. On the following day the cells have become considerably larger (figs. 70, 71) and elongated and somewhat sac-shaped. In

subsequent development (figs. 72, 73) elongation and enlargement of the cells continue and the apical region expands and becomes bulb-like, until in several weeks most of the plants have attained the mature form. I can thus confirm Klebs' contention that the fusing gametes always form a dormant zygote and that the great majority of unfused zoospores can grow directly into new plants. The failure of Rostafinski and Woronin (1877) to observe any further development of the non-fusing zoospores of *Protosiphon* may be correlated with the abnormal conditions in liquid cultures of this organism, which they were employing.

As to the so-called green and red "spores," development is substantially as Klebs and the earlier authors described. Their use of the term "spore," however, for these bodies is unfortunate, for they should more accurately be called "cysts" or "coenocysts." It is well known from the literature of the Protozoa, fungi, and Myxomycetes that encystment stages represent merely a physiological response to a given set of environmental conditions that may appear during the life cycle of an organism, and should this set of environmental conditions not develop, encystment does not take place. De Bary (1887) expressed this conception in his use of the term cyst for those stages of the Myxomycetes which under unfavorable conditions are able to pass into a resting state and to return again to the actively motile phase, when favorable conditions prevail. If we extend the term "cyst" to the algae as apparently West (1916) has done, the red and green "spores" of *Protosiphon* should properly be called "coenocysts," to emphasize their multinucleate constitution. They represent portions of the thallus formed under conditions of drought which can again produce the thallus with the coming of increased moisture content of the substratum. If the coenocysts are placed in water, like the thalli they respond by producing zoospores.

As introductory to a detailed description of the formation and development of coenocysts, zoospores, cytokinesis and fusion of gametes I have illustrated in text figure 1 the life history of *Protosiphon* as observed in cultures. This diagram represents the alternate methods of development and is explained by the legend.

Encystment. As has been reported by all earlier students of *Protosiphon*, under conditions of drought and strong sunlight, the thallus protoplast becomes segmented into a number of spherical coenocysts (fig. 28), which if produced in sufficient numbers become angular by mutual contact and pressure. Their formation is distinctly progressive and may extend over a long period. For this reason it is rather difficult to follow the process in a single thallus, since the plants do not grow well in hanging drops of agar and soon form zoospores if transferred to water cultures for observation.

Figures 25 to 28, a, represent stages of encystment as they appear in

fixed and stained preparations. One or several furrows appear on the outer surface of the protoplast and cut into the cytoplasm in a somewhat curved path (figs. 25, 26). Originating from a point at the inner surface of the thallus wall a membrane projects into the furrow and follows its path through the cytoplasm. This membrane grows rapidly, keeping pace with the progressing cleft (fig. 26). The inner edge of the membrane is often thickened as if an excess of wall forming substance were collected there,

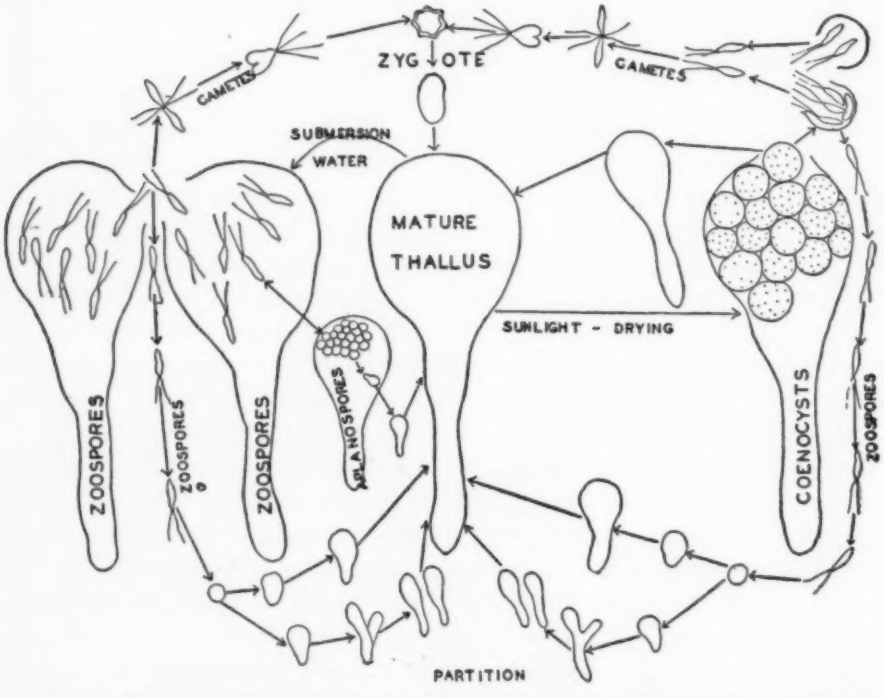

Fig. 1. Diagram of the life history of *Protosiphon botryoides:* thalli or coenocysts when submerged in water produce zoospores which may grow directly into thalli or fuse in pairs forming a zygote which germinates into a thallus after a period of dormancy. Thalli become segmented into coenocysts under conditions of drought and strong sunlight. (See text).

which stains purple in the triple stain. Furrow and membrane thus proceed isolating a portion of the cytoplasm and several nuclei (fig. 27). In large thalli the process is more progressive, larger portions of the protoplast being delimited at first and further subdivided into coenocysts (fig. 25). Although the membrane originates at the thallus wall its substance is doubtless augmented by formation of wall material by the protoplasm on either surface of the furrow.

As the cysts are delimited from the thallus, their protoplasm is highly vacuolate, but as they mature the vacuoles gradually disappear and the cell lumen becomes completely filled with dense, granular protoplasm. This is clearly shown in figure 25, which represents a median section of a thallus undergoing encystment. Encystment of this thallus began at the apex near which the oldest and most mature coenocyst lies; its wall is thicker than that of the others and its protoplasm more dense. The coenocysts at the right of the apical one in the figure, were delimited subsequently and the highly vacuolate mass of protoplasm near the base is just undergoing further subdivision. When several pyrenoids are included in a single coenocyst they tend to collect in the center of the cell. After segmentation of the thallus protoplast is complete, the maturing cysts become densely packed and round off as far as possible (fig. 28). The number of nuclei included in the coenocyst does not increase subsequent to its formation. Mature coenocysts may be further sub-divided as shown in fig. 28. As the cells mature their walls become thickened. Coenocyst formation involves the protoplasm of the rhizoidal portion of the thallus (fig. 28a). This observation makes it questionable whether the basal portion of the thallus should be properly termed a rhizoid in view of Karling's (1932) report that true rhizoids play no role in multiplication of the thallus in the fungi.

Zoosporogenesis in the thallus. As has been noted above, Cienkowski (1855), Rostafinski and Woronin (1877) and Klebs (1896) describe the formation of zoospores from thalli growing on solid media when covered with water. This occurs in nature when the soil is saturated after heavy rains or floods. It has been my experience that swarmspore formation is the invariable response to submerging plants from agar cultures. It is usually difficult to secure zoospores from plants younger than twelve to fourteen days, but after this time almost every submerged thallus undergoes zoosporogenesis.

The following method was used for securing and studying zoospore formation: Large plants from month-old cultures were carefully removed from the agar and placed in hanging drop cultures of distilled water or dilute (.57%) Detmer's solution. At the same time the plants which remained in the culture were submerged to a depth of half a centimeter. The hanging drop mounts were retained under constant observation while thalli from the submerged agar cultures were periodically observed as a check on possible pathological conditions which might develop in the drop cultures.

A large number of thalli undergoing zoosporogenesis have been studied and the observations here reported are based on those of March 19, 1932.

Plants from a culture of February 18 were placed in hanging drops of distilled water and the plants remaining in the culture were submerged as described above. Several cultures were kept under observation simul-

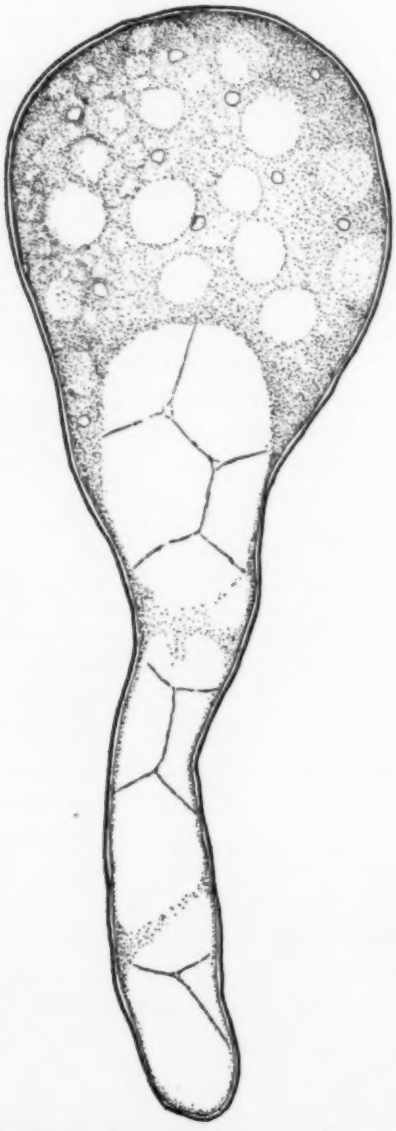

Fig. 2. Appearance of thallus immediately after submersion in water (11:45 A.M.).

taneously and although text figures 2, 3, and 4 are drawings of the same thallus at successive stages, they are typical of all thalli.

Text figure 2 illustrates the appearance of the thallus at 11:45 A.M. immediately after immersion in the hanging drop. Most of the protoplasm lies in the upper portion of the cell, while a thin peripheral layer extends over the inner surface of the wall toward the rhizoidal region. The chlorophyll is limited to the cytoplasm of the expanded region of the thallus leaving the basal region quite colorless. The chlorophyll-bearing cytoplasm is not sharply bounded from the colorless. At the cell base the protoplasmic layer or primordial utricle is extremely thin and the vacuole extends very close to the wall. The protoplasm at the apex of the cell is extremely spongy and vacuolate and the hyaline pyrenoids and numerous small oil droplets are conspicuous. The center of the cell is occupied by a large vacuole which in this particular cell is traversed and subdivided by strands of cytoplasm into several chambers of bubble-like appearance. This chambered condition of the central vacuole does not occur generally and has been only occasionally observed. Within thirty minutes the protoplast became less spongy and more densely granular. This change appeared to come about by the gradual excretion of the more aqueous phase of the protoplasm into the central vacuole, as evidenced by changes in the shape of the chambers, movements of their bounding membranes, and the appearance of oil droplets and granules in the previously optically empty vacuole. The protoplasm near the rhizoidal pole flowed gradually towards the apical region. By 2 P.M. the vacuolated regions in the protoplast were very much less evident, causing it to appear densely green and granular. Yet at this time the cell was still completely turgid. At 2:10 P.M. contraction began, the protoplast shrinking from the apical region of the cell wall, and at the same time the vacuole began to draw away from the base of the rhizoid. The pyrenoids were still visible at this stage but immediately after became obscured due to the increasing density of the chlorophyll-bearing cytoplasm. This increasing density is the result of further dehydration of the aqueous phase of the protoplast. Small vacuoles with refractive droplets were continually forming and bursting from the outer surface of the protoplasm as well as discharging into the central vacuole, or in some cases remained attached to the surface layers for some time. The formation and bursting of these surface vacuoles appears very similar but less rapid than the action of the contractile vacuoles described by Lloyd (1924, 1926 a, b) in the contraction of the gametes of *Spirogyra* prior to conjugation.

The contraction phase occupied two hours and was of relatively long duration compared with the other stages in cleavage. The extrusion of liquid from the surface of the protoplast and central vacuole continued as far as I could observe until 4 P.M. During this period there was constant

change in size and shape of the chambers of the central vacuole. By 4 P.M. the protoplast had shrunken to approximately one-third its original volume, and the central vacuole was but a small sac filled with numerous

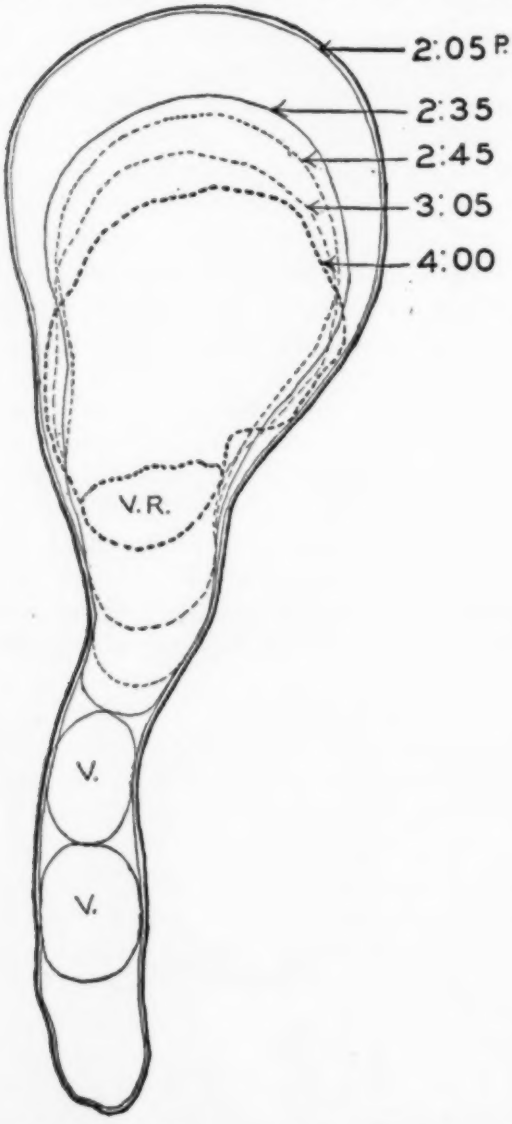

Fig. 3. Successive stages of contraction of the protoplast in thallus of fig. 2. (time intervals indicated). V.R. = vacuole remnant.

droplets and granules. Text figure 3 represents changes in the outline of the protoplast and its relation to the wall in optical section from the

initiation to the completion of contraction. The changes of position with reference to the cell wall during contraction become apparent from these diagrams. Of special interest is the relation of the plasma membrane to the wall during this gradual contraction. As the protoplast drew away there remained numerous fine hyaline threads running from the surface of the protoplast to the cell wall which persisted until the protoplast had shrunken to about one-half of its original volume. I have been unable to follow with certainty the fate of these threads in living cells but their presence here is highly suggestive of the observations reported by Chodat and Boubier (1888) that in slow, artificial plasmolysis of plant cells the plasma membrane remains connected to the wall by hyaline strands. The plasma membrane was very clearly visible as a double contoured structure during the contraction phases.

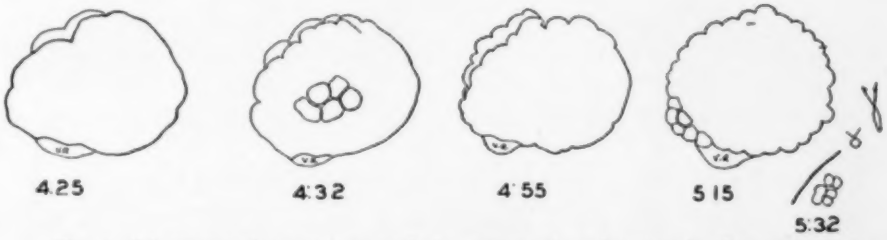

Fig. 4. Diagrams of zoosporogenesis by progressive cleavage in the same protoplast: (time intervals indicated).

From 4 to 4:10 P.M. no visible change of the protoplast occurred. At 4:10 P.M. the rounded contour of the protoplast began to change rapidly; the surface of the protoplast became slightly folded and lobed and in optical section the surface seemed to be invaginated at several places. Following this lobing more numerous delicate invaginations or furrows appeared over the surface. This process of furrowing began first at one pole of the cell and extended gradually over the entire surface (text fig. 4). The course of the furrows was irregular, curved and branched and they appeared as narrow, linear, hyaline areas traversing the dense protoplast. It was impossible to follow their course more deeply into the protoplasm because of its density. These furrows continued to extend and branch until they had delimited large blocks of cytoplasm. By 4:45 P.M. the entire surface was cut up into these large blocks which showed a slight tendency to polygonal outline. Careful focusing showed the formation of new furrows on the surface of these large blocks which by 5:25 P.M. had subdivided the protoplast into numerous small segments.

Then followed a period of slight swelling so that the surfaces of the re-

cently delimited segments became closely appressed and the lines of cleavage more difficult to follow. However, a study of the margin of the protoplast demonstrated that the latter was completely segmented. Superficial segments observed at this time appeared to be undergoing bipartition, a lighter hyaline area forming across the equator of each segment.

At 6:03 P.M. a slight gliding movement of the segments began which became gradually accelerated until they developed a quite rapid motion. At the beginning of the motile period none of the segments moved outside the original boundary of the contracted protoplast, but with the acceleration of motility they finally invaded the entire lumen of the thallus. As movement increased it changed from a slight local turning and gliding motion to a more active darting back and forth of the swarmspores. These motile segments which were first confined to within a short radius of their place of origin moved to greater distances away from it. I am at a loss to account for the tendency of the motile bodies to remain at the center of the thallus cavity for some time after motility has begun, since there is no sign of any gelatinous material or bounding membrane to hold them in position. The nature of their surfaces may possibly cause them to adhere to each other.

During this period of motility within the thallus the zoospores increased slightly in size and changed their shape from irregular ovoid to somewhat definitely heart-shaped and finally became quite elongated and spindle-shaped (text fig. 4). The first zoospores were liberated at 7:10 P.M. by a localized, apical, slightly lateral rupture of the mother wall. At first they escaped a few at a time and rather slowly as if they were passing through a gelatinous layer which inhibited rapid motion. The remaining individuals swam rapidly through the opening into the surrounding medium. Several were apparently unable to escape from cell lumen and continued their movement until observation ceased. The entire process of zoospore formation from the immersion of the cells in water to the liberation of the mature swarmspores occupied in this particular thallus seven and three quarter hours.

Although the above description applies in general to all cleaving cells of *Protosiphon*, I have noted several slight modifications. In certain thalli during contraction the protoplast may become divided into two or several portions which are separate from each other but partly in contact with the central vacuole. In a very few cases cleavage appeared to begin before contraction became at all extensive. In such cases the protoplasm is more vacuolate and the furrows appear broader, some of them appearing to be almost of vacuolar nature. The period required for contraction and cleavage varies somewhat with the size of the cell, being in general more rapid

in the smaller thalli. This apparent correlation can be more advantageously discussed at the conclusion of the following section.

It is obvious from the above account that only the more general features of the cleavage process can be observed in living cells. For details month-old cultures were submerged in water and weak Detmer's solution and pieces of the agar with the plants were cut out and fixed at various time intervals, imbedded and sectioned at from five to ten microns as described above.

Such sections of cells which had been fixed immediately after immersion in water have the appearance of the one shown in figure 1, which is a slightly oblique section of a thallus. The rhizoidal portion of the cell here shown is completely filled with protoplasm but study of the consecutive sections demonstrated that this is largely due to the orientation of the thallus in such a manner that the plane of section cut through the periphery of the rhizoidal region and formed a more median section at the apex of the thallus. Median sections of the cell base (figs. 2, 3) show that the protoplasm normally consists of a thin parietal film between the wall and central vacuole. In the bulbous portion the primordial utricle is much thicker and the vacuole is much smaller in proportion to the volume of the cell cavity. The protoplasm is an extremely loose and spongy reticulum as in the living cells and the nuclei lie within its meshes. The number of nuclei in a cell of the size shown in figure 1 is very great in view of the fact that nuclei here figured consist only of those lying in a single plane of focus ten microns thick. The tonoplast is quite conspicuous as a thin blue-staining membrane between the vacuole and protoplast and the plasma membrane stains in similar fashion.

Although some plasmolysis occurs in fixation it can be readily distinguished from the shrinkage which occurs in preparation for cleavage. In the latter the protoplasmic reticulum is less open, while in the former the shrinkage is apparently confined to slight loss of water from the central vacuole, leaving the remainder of the protoplasmic structure unaffected. The suggestion based on observation of living cells that shrinkage is accomplished by extrusion of water and droplets of material from the protoplast into the central vacuole as well as from the periphery is readily confirmed in the study of fixed sections (figs. 29, 31, 32). Many such preparations show that material is accumulating in the central vacuole near the tonoplast and that vacuoles are forming and bursting at the surface. Some of this material is in the form of droplets, while in many cases there is also an abundance of numerous small granules which have great avidity for gentian violet.

During contraction the plasma membrane is conspicuous (figs. 29–31).

In the most favorable preparations it stains deeply with gentian violet and is clearly distinguishable from the deeper cytoplasm which shows affinity for Orange G. As soon as contraction of the protoplast has been completed cleavage begins. Division of the protoplasm is clearly accomplished by a process of progressive cleavage by furrows and not by either successive bipartition or so-called simultaneous multipartition by precipitation of membranes between the nuclei. This fact becomes very obvious from examination of preparations such as those figured in 29–32, 29 and 31 being cross sections through the contracted protoplast at right angles to the long axis of the thallus, while 32 represents a slightly oblique section. Only a portion of the thallus wall is shown in these figures but it is nonetheless sufficient to indicate the extensive shrinkage of the protoplasm. The cavity between the wall and surface of the spore plasm is completely void of any stainable material and appears as an optically empty region even in the most densely stained preparations. The only exception to this statement is the occasional occurrence of droplets and granules between the wall and protoplast, presumably secreted by the latter (fig. 37).

The furrows may begin either internally from the surface of the vacuole or at the periphery by invagination of the plasma membrane (fig. 29). From the study of successive stages of the process it becomes evident that furrows may also appear in the deeper protoplasm simultaneously with surface furrows. These more deeply lying furrows appear to terminate abruptly and are not continuous with those from the surface. These later clefts are open and broad at the surfaces but narrow as they cut deeper into the protoplasm and have sharp edges. As noted by Harper, there is no visible differentiation of the protoplasm in the path of the progressing cleavage furrow. The furrows cut through the spore plasm at varying angles and with curved paths at first with no apparent relation to the nuclei or to each other. Usually from the initiation of the contraction stage to the completion of cleavage no nuclear divisions take place (figs. 29–33).

As emphasized in the study of living cells, the surface furrows may form and progress more rapidly in one region than in others as shown in figure 29. This figure illustrates clearly the tendency for furrows from the vacuolar surface to fuse with centripetal radial furrows from the plasma membrane as is the case in *Didymium*. By the gradual coalescence of these and further tangential clefts large irregular multinucleate masses are cut out as illustrated in figures 29,31,32. Furrows then appear on the surfaces of these multinucleate blocks which are finally divided into uninucleate segments as shown in figure 33. During these final stages of cleavage the nuclei tend to lie near the path of the cleavage furrows.

These segments are relatively small and numerous and closely ap-

pressed as shown in figure 33, which represents a small portion of a section. The more central segments are often more polygonal in shape than the peripheral ones. The single nucleus is in the resting stage which is typical during the entire cleavage process (fig. 30). Although in many cases the uninucleate segments are closely appressed, it is not very difficult to observe the clefts between them; these segments are destitute of cell walls and are bounded merely by their plasma membranes.

The cleavage segments do not become motile immediately after their formation, and they are not definitive swarmspores, swarmspore-initials or swarmspore anlagen. As far as my observations go they will under no circumstances develop flagella and function as swarmspores. In the discussion of the process in living cells evidence of a bi-partition of the end products of cleavage was pointed out. This occurs before motility is initiated. Study of stained sections confirms this observation. The single nucleus of every segment undergoes division (fig. 34) which is then followed by bipartition of the segment (fig. 35, 36). The nuclei of the segments throughout the entire thallus divide with almost complete synchrony. Cell division is accomplished by the formation of a lighter staining zone across the equator of the now bi-nucleate segments (fig. 36), through which constriction takes place. I am still uncertain as to whether one or two nuclear and cell divisions occur in the uninucleate protoplasts. An attempt to secure indirect evidence on this question by measuring the relative sizes of the constricting segments, and comparing them with the initial uni-nucleate protoplasts was abandoned because of the great variation in the size of the original segments themselves. The question as to whether more than one division regularly occurs thus remains unsettled. At the conclusion of the final bi-partition the segments withdraw somewhat from each other, increase slightly in size and develop into swarmspores.

The nucleus enlarges slightly and takes up a peripheral position so that the nuclear membrane is in close proximity to the plasma membrane (fig. 46) of the zoospore initial. Immediately after this stage there appears a deeply staining granule between the nucleus and the plasma membrane from which the flagella develop (figs. 37, 45, 46). I have been unable to trace the exact origin of this granule. It always arises in close association with the nucleus and is unquestionably the basal body or blepharoplast from which the flagella are organized. Its close association with the nucleus suggests the possibility that it may have been derived from the centrosomes which are present at the poles of the spindle figure.

Shortly following this stage the zoospore initials separate, doubtless as a result of the motility described above in living material, and their slight

increase in size and change in shape can be readily followed in the sectioned material (figs. 37, 45–47). In free swimming zoospores (figs. 47, 53) the nucleus lies near the apex and slightly withdrawn from the plasma membrane. In favorable preparations it is clearly seen to be connected to the basal body by a delicate protoplasmic fiber or strand (rhizoplast). The chromatin appears more condensed in the nuclei of the free swimming zoospores but it is possible that this is only the result of the method of preparation. The stigma does not stain clearly. In some of the zoospores one or several small pyrenoids are present in the posterior region, while others lack them (figs. 67, 68).

As to the behavior and development of the pyrenoid during zoosporogenesis there is some variation. In living cells it soon becomes obscured by the increasing density of the protoplasm, while in stained preparations it sometimes appears to break down into smaller fragments (figs. 29, 31) which may be disseminated through the cleaving cytoplasm or remain collected in clear areas (fig. 31). In other cases no trace of fragments can be found (fig. 32) and the pyrenoid loses its affinity for the stain, leaving a clear area in the cytoplasm. In the former case most of the zoospores receive one or more of the red staining fragments during cleavage, while others lack them entirely. When the pyrenoid entirely disappears new ones arise *de novo* in the zoospores after they have come to rest. This is quite evident from living zoospores (figs. 67, 68), some of which have pyrenoids and others not. The data on the question of the inheritance of the pyrenoid has been summarized in a previous paper (Bold, 1931); pyrenoid behavior in *Protosiphon* is similar to that described by Miss Carter (1926) for members of the Ulvaceae. The contractile vacuoles in well fixed preparations appear as clear areas at the anterior extremity of the cell (figs. 47, 53).

Zoosporogenesis in coenocysts. Zoospore formation has been repeatedly observed in both green and red coenocysts of *Protosiphon* after removing them from agar cultures or soil to drops of liquid media as in the case of the thalli. Text figures 5, 6, and 7 are diagrams of this process as it occurs in living cells. In all essentials it is similar to that described in the thalli. In young coenocysts with vacuolated protoplasm the protoplast becomes denser and collects at one side of the vacuole (text fig. 5) by the progressive excretion of water, droplets, and granules into this latter region. As the protoplasm extrudes water its vacuolate appearance changes and the pyrenoid becomes obscured with the increase in density. Loss of water from the protoplast and central vacuole continues until the volume of the protoplast has greatly decreased (text fig. 5). When contraction has reached a certain point segmentation is initiated. It is accomplished by invaginations or surface furrows which grow and branch, isolating some-

what irregular masses of protoplasm. These are in turn sub-divided by secondary furrows on their surfaces into still smaller portions. As in the case of the mature thalli these segments swell slightly, become closely appressed, and remain in this condition for a short time. Finally they undergo either one or two bipartitions, so that there are formed in the coenocyst cavity a number of small protoplasts. These round off and develop flagella. A feeble gliding movement becomes evident and is gradually increased until the swarmspores are swimming rapidly through the cavity of the coenocyst. They are liberated by rupture of the wall and swim away

Fig. 5. Diagrams of zoosporogenesis in immature, vacuolate coenocysts.

into the culture medium. I have not so far observed the occasional liberation of zoospores in a gelatinous vesicle which Klebs figures; however, this method of escape is not universal as he has pointed out. The central vacuole often remains within the empty cell as a small hyaline sphere, which indicates a well differentiated and persistent tonoplast.

In more mature coenocysts in which the central vacuole has disappeared entirely and whose cell cavity is completely filled with dense protoplasm, cleavage is more rapid, and practically no shrinkage occurs

Fig. 6. Diagrams of zoosporogenesis in mature coenocysts with dense protoplasm in which the central vacuole has disappeared.

(text fig. 6, figs. 40, 43, 44, 48). Very soon after immersion in water furrows are formed at the surface of such cysts which quickly separate the protoplast into large segments which are subsequently divided into smaller portions. The final bipartitions take place as in the vacuolated cysts. The process of cleavage is thus the same in young vacuolated coenocysts as in the older, densely filled cells with the exception that it is more rapid in the latter type and a larger number of zoospores are produced in proportion to the cyst size.

I have already described the formation and structure of the coenocysts as they appear in sectioned and stained material. In younger cysts as in

the thalli the protoplast is spongy and vacuolate (figs. 38, 39) but during zoospore formation liquid is extruded into the central vacuole and it therefore becomes denser in appearance. In fixed and stained preparations it is apparent that the contraction stages are due first to the extrusion of water and other materials into the central vacuole and from the surface of the protoplast, and secondly to the decrease in volume of the vacuole itself (fig. 41). Except in the smallest cysts and aplanospores segmentation is invariably a process of progressive cleavage. When contraction is completed furrows appear in the protoplasm. In larger coenocysts the furrows may appear earlier at one pole than at the other so that cleavage may progress more rapidly in one part of the cell than in the other. Later clefts form in the deeper cytoplasm. They are usually broader at the surface and thin out at their edges (figs. 41–43). They curve and fork in various directions and as they come together isolate small multinucleate regions of the protoplast. In small coenocysts (text fig. 7) the primary furrows delimit

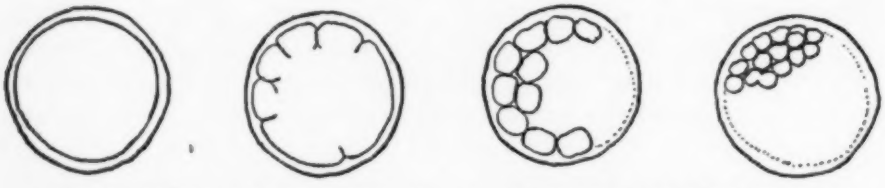

Fig. 7. Diagrams of zoosporogenesis in small coenocysts in which the primary cleavage furrows delimit uninucleate protospores directly.

uninucleate segments directly (fig. 43). In larger cysts when the multinucleate segments have been formed they are also sub-divided by furrows so that the protoplast of the coenocyst is transformed into a number of uninucleate segments. During the final cleavage stages the nuclei lie very near the cleavage planes and usually retain this position during the further development of the uninucleate segments (fig. 44). As in the thalli these uninucleate segments are not the zoospore initials or anlagen. After a period of slight swelling the single nucleus divides either once or twice (figs. 48, 51) and then follow one or two bi-partitions (fig. 50), the products of which become the zoospores directly and without further division. These segments round off somewhat and a blepharoplast appears near the plasma membrane from which the two flagella develop. When they first become motile they increase slightly in size and are globose, but as their movement is accelerated they become spindle-shaped (fig. 47). They are liberated by rupture of the coenocyst wall.

The process of cleavage is the same in both red and green coenocysts whether newly formed or mature, with the exception that in mature cysts

it is more rapid and there is very little contraction (figs. 40–44, and text fig. 6). In small cysts with few nuclei the process is similar but abbreviated (text fig. 7)

As the cysts vary in size they vary in the number of zoospores they produce. I have so far not observed the escape of less than four zoospores from a single coenocyst and this happens only occasionally. Usually the number is larger and in those cysts whose zoospores I counted carefully the number was almost invariably a multiple of two, presumably as a result of the final bipartitions

Structure and further development of the zoospores. When a large number of thalli and coenocysts of varying stages of maturity are placed in water, myriads of zoospores are produced within several hours and continue to be liberated for periods as long as several days. Liquid cultures thus become actually turbid with the swarms of zoospores which are positively aero- and phototactic, collecting on the surface of the medium nearest the source of light. If such a culture is rotated 180° with reference to the light source the zoospores move in a dense green cloud back towards the light. Motility appears to be more rapid in bright light than in the dark.

The zoospores are somewhat variable in both size and shape (figs. 67, 68). A few attain the length of 11 microns while the majority vary between 3 and 6 microns. When first liberated they may be somewhat globose, but as they swim about they gradually become elongated and spindle-shaped and in end view (fig. 68, a) appear round. Both ends are often colorless and pointed and a hyaline droplet is occasionally attached to the posterior end (fig. 47). The chlorophyll may extend to the posterior end but the anterior portion is always hyaline. In the living cells the blepharoplast with the two attached flagella can be quite clearly distinguished. The flagella are approximately as long as or slightly longer than the length of the cell body and beat very rapidly in propelling the zoospore. A minute pyrenoid may or may not be present but all zoospores contain a red eye spot which is imbedded in the green portion of the cytoplasm near the anterior margin. Two contractile vacuoles lie immediately below the blepharoplast. There is no conspicuous, definite cell boundary in addition to the plasma membrane. The plasticity of the zoospores becomes apparent when they collide with each other or force their way between other cells. They tend to collect near the margin of hanging drops and as they come to rest other zoospores swim towards the margin and force their way between them, and as this continues they become angular by mutual compression and contact (fig. 67, i). This occurs also on solid media and in such cultures the zoospores continue to develop so that

that pole of the cell not in contact with the substratum begins to enlarge in a direction perpendicular to the surface of the culture medium. In densely inoculated cultures as a result of the aggregation of the zoospores the bulbous aerial portion of the thallus becomes more slender and tapering (fig. 4). I have described previously (1931) a similar aggregation of the swarmspores as they come to rest in *Chlorococcum infusionum*. The polyhedral cell shape which is assumed in this organism, as the zoospores settle in crowded groups, is more permanent, resulting in loose coenobia of angular individuals, but isolated cells develop the spherical shape. In *Limnodictyon Roemerianum* Ktzg. on the other hand (which is apparently closely related to *Chlorococcum*) this polyhedral shape, presumably acquired in the same way, has become fixed, since the cells of this species do not resume the spherical shape even when separated from the coenobium, as they do in *Chlorococcum*. In my first paper I suggested that the regularly constructed coenobia of *Hydrodictyon* and *Pediastrum* species may have developed from some such simple type of aggregation as occurs in these genera, and it is significant to note that Jost (1930) reports that isolated zoospores, mechanically freed from the *Hydrodictyon* mother cell, tend to develop the cylindrical cell shape when lying free in the culture medium.

Among the large numbers of zoospores liberated from cultures submerged in water many conjugating pairs are readily visible (fig. 67). In the process of fusion two individuals approach and become entangled with their flagella. They may swim around in this way for long periods (more than an hour) and finally separate. More often, however, when two zoospores become attached they begin to fuse at the anterior ends. In most of the cases observed the process occupies about one hour, and during this period the fusing pair continues motile. As far as can be determined in the living, motile cells, fusion begins at the anterior point where the blepharoplasts lie (67, a) and as it continues the cells appear to bend around toward each other (67, c–f), fusion proceeding gradually from the anterior to the posterior end. The completely fused gametes may continue motile for a short time and during this period they exhibit the same positive phototactic and aerotactic responses as the zoospores, intermingling with the latter at the margin of the drop (fig. 67, i). The outlines of the individual fused protoplasts are still distinct for some time after the zygote has come to rest and the stigmas and contractile vacuoles are present in double number, but eventually disappear (figs. 67, g, i).

The conjugation of zoospores has also been followed in fixed and stained preparations and the process is illustrated in figures 53 to 61. The gametes first come in contact at their anterior poles in the vicinity of the blepharoplasts (fig. 53) in the manner described for living material. Fusion is thus

accomplished by gradual coalescence along the adjacent lateral surfaces of the two individuals beginning at the anterior and extending progressively toward the posterior pole. The two nuclei retain their position near the blepharoplasts and may sometimes be in contact, while the blepharoplasts are very closely appressed and often appear as a single bi-lobed structure (figs. 54, 57, 58). After coming to rest the zygote becomes more spherical and the nuclei appear to lie nearer the center.

It is obviously impossible to follow the complete process of gamete fusion in the same individual pair in fixed and stained material, but some data have nonetheless been obtained on the length of time required for this process. A large number of thalli and cysts were placed in a small vial of water and carefully examined from time to time. Immediately after the first gametes and zoospores appeared near the surface of the medium fixations were made repeatedly at hourly intervals. The preparations fixed during the first two hours showed no zygotes with fusing nuclei, but did show many which had come to rest and whose nuclei were still separate. Material fixed during the third hour contained a majority of zygotes in which the nuclei were still separate, but in a few the nuclei were fusing or had fused completely. Preparations during the fourth and subsequent hours contained numerous zygotes in which nuclear fusion was taking place or had been completed. Such data are of course not specific for individual zygotes but nevertheless suggest that nuclear fusion in general takes place between three and four hours after liberation of the gametes and their plasmogamy.

The preparations of fusing gametes were made by the smear method and stained with haemtoxylin and the triple stain; although nuclear details are quite clearly preserved the cytoplasm tends to stain densely, making it difficult to follow the fate of the blepharoplasts and flagella. In syngamy the nuclei come to lie in contact in the more central cytoplasm (fig. 59). They appear to enlarge slightly before fusion but in contrast to the nuclei of the free swimming zoospores they do not stain densely. The nucleoles are clearly differentiated and the chromatin is distributed in the form of delicate strands within the nuclear cavity. The nuclear membrane eventually disappears between the two nuclei and fusion takes place (fig. 60). The zygote nucleus is approximately double the size of the gamete nucleus and for some time later the two nucleoli remain distinct, while the chromatin contributed by each gamete nucleus becomes indistinguishably dispersed through the nuclear lumen. The two nucleoli often persist during the dormant period of the zygospore (fig. 62). The nucleus of the dormant zygospore is similar in position and structure to that in the young ones. Although I have so far been unable to follow definitely the

fate of the blepharoplasts in the zygote because of the density of the cytoplasm, some preparations occasionally showed rather deeply stained granular material in contact with the nuclear membrane (fig. 60) and it is possible that this is the remnant of the blepharoplast. The fate of the flagella in zygotes and zoospores could be readily traced in living cells. In either case they do not drop off immediately but continue to beat feebly for a short time. With this feeble beating they become gradually shorter and are then withdrawn into the cell. I have not succeeded in differentiating them after they are withdrawn into the cells in fixed preparations, but my observations of living cells convince me that they are not sloughed off but drawn back into the protoplasm (fig. 67, g–i).

In spite of the variation in size of the zoospores it is impossible to find any marked tendency for the larger gametes to conjugate with the smaller. Instead it has been my observation that the gametes which fuse are more often of similar size, large zoospores fusing in pairs as often as small ones. However, some cases of large gametes conjugating with small have been found (fig. 67, b).

Miss Carter (1926) in a footnote (p. 682) has noted that the gametes of *Protosiphon* will fuse and form zygotes within the same cell before they are liberated. Klebs (1896) on the other hand states: "Dagegen konnte ich noch feststellen, dass die Schwärmer der gleichen Mutterzelle nicht miteinander kopulieren." In view of the recent accounts of Hartmann (1929), Föyn (1929), and Gross (1931) of heterothallism among the Chlorophyceae and Schussnig's (1930, 1931) report of sex chromosomes in *Cladophora*, careful attention has been paid to this point in my study of *Protosiphon*. Numerous single thalli were removed from agar and placed singly in hanging drops to secure swarmspore formation. In a similar way single thalli which had formed coenocysts were isolated in hanging drops and finally single coenocysts themselves. In such single-thallus and -coenocyst cultures I have so far not observed the fusion of two zoospores. Two individuals may become attached for some time but finally they separate, and develop directly into new plants when placed on nutrient agar. In contrast conjugation is widespread in cultures containing numerous thalli. As noted above it often happens that several zoospores fail to escape from the thallus wall after zoosporogenesis and remain in the parent cell, come to rest and begin to develop. In examining many such thalli I have occasionally found a single star-shaped zygospore within the old thallus wall. Miss Carter's conclusion that *Protosiphon* is "monoecious" was based on the discovery of fusing isogametes within the same thallus resulting in typical zygospores. Klebs reports a few similar observations. Although such cases undoubtedly occur, my observations agree with Klebs' and indi-

cate that as a general rule gametes from the same thallus or coenocyst do not fuse with each other. I have so far obtained no conclusive evidence to affirm or deny the existence of genotypic differences in the gametes of *Protosiphon* and the cells which produce them.

Germination of the zygospore. As has been noted above the zygospore undergoes a period of dormancy. When a number of zygospores are transferred from an old, dry culture to fresh media their contents swell and the thickening of the walls becomes less apparent as the cells increase in size. After the cell size has increased somewhat and the zygotes have become more regular in outline, their growth is greater along a single axis and they soon become sac shaped and germinate into typical thalli. As yet I have not secured a complete series of fixed preparations of the germination of zygospores but certain stages appear significant. As the protoplast swells the single nucleus which has remained in the "resting" condition during dormancy, increases in size and the chromatin becomes aggregated into rather thick masses unevenly distributed within the nuclear cavity (figs. 63, 64). Subsequently some of the zygospores become binucleate (fig. 65) and finally tetra-nucleate (fig. 66). In the majority of cases by the time the tetra-nucleate stage is reached the contour of the cell is quite smooth. The nuclei continue to divide and the cell increases in size, eventually assuming the typical shape of the thallus. On the basis of these observations of the nuclear changes in the germinating zygote it is not yet certain whether or not reduction takes place. Too many of the intermediate and critical stages such as synapsis, diakinesis and careful chromosome counts are lacking to definitely answer this question. One might draw an analogy on the basis of the evidence at hand with conditions described by Miss Gross (1931) in *Ulothrix* and Allen (1905) in *Coleochaete* and suggest that reduction may occur in the germination of the zygote, but the evidence so far obtained is insufficient. A further report bearing on this question will be published as soon as more conclusive evidence is obtained.

DISCUSSION

As early as 1858 DeBary reported in his "Untersuchungen über die Familie der Conjugaten" that starch grains were present around the pyrenoids in *Spirogyra*. The association of starch grain formation with pyrenoids was emphasized by Schmitz (1882) who was the first to use the term pyrenoid. It has since been observed by many investigators, but it remained for Timberlake (1901) to demonstrate by refined staining technique that the large number of small autochthonous[1] starch grains in the

[1] Term first used by Wiesner (1900).

cytoplasm of *Hydrodictyon* cells are but transformed segments of the pyrenoid body, which have been split off from the latter and then been pushed out into the cytoplasm. The pyrenoids were commonly described and figured as spherical but in many cases they appear markedly polygonal in section as noted by Meyer (1883) in *Spirogyra;* this shape was not however viewed as related to the formation of the surrounding starch bodies by the earlier investigators. Timberlake showed that this characteristic sectorial contour came about by the splitting off of successive tangential segments from the mass of the pyrenoid which were gradually changed into typical starch grains. According to his account the transformation of such proteinaceous pyrenoid sectors into starch is accompanied by successive changes in staining reaction as the segments are pushed further out into the cytoplasm. Progressing centrifugally from the pyrenoid to the outermost segments in the cytoplasm, Timberlake described a gradual, transitional staining reaction from red, to gray, to purple, and finally to blue. The outlying segments stain characteristically blue in gentian violet and give the typical starch reaction with iodine.

Since the publication of Timberlake's observations several papers have appeared on the relation of the pyrenoid to photosynthesis in algae. Lutman (1910) in his study of *Closterium* found a single layer of large starch grains surrounding the pyrenoid in the form of a hollow sphere. Although the pyrenoid body was often much cracked and in some cases in planes which corresponded to the spaces between the starch grains, Lutman found no convincing evidence of the direct transformation of the pyrenoid segments into starch grains, such as Timberlake found in *Hydrodictyon*. McAllister (1914) and more recently his student Miss Ma (1931a, b) in their studies of the "multiple pyrenoids"[2] of *Anthoceros*, *Selaginella* and certain mosses have discovered that the elements of these pyrenoids show a change in their staining reaction successively from the center outward from brilliant red to purple to blue when treated with the Flemming triple stain, and they come to the conclusion that the elements of the multiple pyrenoid are the primordia of the starch grains. From my observations of *Chlorococcum* (1931) it appears that segments are cut off from the pyrenoid, become dissolved in the hyaline zone and subsequently starch is deposited in the cytoplasm surrounding the hyaline zone. On the other hand Bourquin (1917) reports that in *Zygnema* starch is formed at the periphery of the chloroplast without visible relation to the pyrenoid.

Study of the pyrenoids and their relation to starch formation in *Proto-*

[2] Term first used by McAllister (1914) to describe the compound condition of the pyrenoid in *Anthoceros*.

siphon leaves no room for doubt that the process is essentially similar to that which Timberlake described in *Hydrodictyon*. In *Protosiphon* the pyrenoid body stains brilliantly with safranin and surface segments separate from it. Both before and after they have completely separated they tend to stain a reddish-purple color, staining more densely than the pyrenoid itself, and as they move out into the cytoplasm they are transformed into typical blue-staining starch grains. Thus there is further evidence in *Protosiphon* for the view that starch formation at least in forms with pyrenoids involves the chemical dissociation of protein pyrenoid material into the carbohydrate starch. That a similar relation between protein and starch may exist in organisms without pyrenoids is suggested by the cytological work of Ma (1931b) on mosses, and by the chemical work of Samec and Haerdtl (1920) and Taylor and Walton (1929). Their report of the presence of phosphorus in starch has never been adequately explained on the current theory as supported by Willstätter and others that starch is the direct product of a synthesis of carbon dioxide and water, through such stages as formaldehyde and sugars. In addition Taylor and Nelson (1920) report the presence of nitrogen in the starch grains of the higher plants.

In relation to current theories, Baly, Heilbron, and Barker (1921) report the formation of formaldehyde from aqueous carbon dioxide solutions in light of short wave lengths, and the formation of sugars from formaldehyde in visible light when suitable photocatalysts were employed, and on the basis of their experiments suggest that the process of photosynthesis as it occurs in the green plant is largely dependent on the efficiency of chlorophyll as a photocatalyst. In accordance with such views it is generally assumed that the typical plant metabolism, like that of animals, consists essentially in the breaking up of complex carbohydrates as sources of the energy involved in the vital processes, the photosynthetic process being regarded as a preliminary, and as it were, an extraneous manufacturing of food materials.

There is no adequate explanation, however, on the basis of current theories of photosynthesis, for the observed fact that in organisms having chloroplasts with pyrenoids, photosynthesis involves a transformation of protein into starch or possibly sugars. Observation of the method of starch formation in such forms suggests rather that carbohydrate formation is a phase of the anabolic processes, involved directly in the building of the protoplasm of the green plant. The relation of the pyrenoid to starch formation suggests that the latter is in reality a storage product and that the protein of the pyrenoid is first formed as an excess in the general anabolic synthesis of food salts, carbon dioxide, and water into protoplasm. Starch

is subsequently formed as a storage product by the dissociation of the protein of the pyrenoid. The view that starch is a dissociation product of the proteinaceous pyrenoid is in harmony with Vines' (1886) theory of photosynthesis in the higher plants, which he compares with Strasburger's (1882) view that the cell wall arises by the transformation of peripheral protoplasm into wall substance. Vines maintains that photosynthesis involves the constructive metabolism (anabolic phase) of protoplasm, and that starch is to be regarded as a temporary reserve material formed as a dissociation product of protoplasm after protoplasmic synthesis has taken place. Vines emphasizes in his theory (thus deviating from current theories), the intimate chemical relation of the constructive metabolism of protoplasm with photosynthesis. His theory is supported by the data of Spoehr and McGee (1923) who found a close correlation between the rate of photosynthesis and the rate of respiration of lighted plants, so that decreased supply of carbohydrates in the leaf results in decrease in both photosynthesis and respiration. In this connection a statement of Miller (1931, p. 440) is significant in which he says: "In regard to the question concerning the first sugar formed in photosynthesis, an observation of Miller (1924) is worthy of note. He found that the total increase in the dry matter of the leaves during the day could not be accounted for by the increase in the sugars and insoluble carbohydrates during the same period. The increase in the total sugars and insoluble carbohydrates in the leaves during the day approximated only from 46 to 92 per cent of the total increase in the dry weight of the leaves for the same period. Since the cellulose content of the leaves did not increase during that period, it would appear that certain compounds were being formed which are not detected by the methods of carbohydrate determination as now used."

Coenocyst formation in *Protosiphon* is strikingly similar to cytokinesis in many other algae. *Cladophora* has long been a favorite object for the study of cell division. As early as 1835 Winter, and later Von Mohl (1845) described and figured quite accurately cell division in *Cladophora* (*Conferva*) *glomerata*, by a so-called ingrowth of a ring-like partition from the wall, which divided the protoplast of the mother cell, and thus for the first time proved that cells arise by division from pre-existing cells. On both surfaces of this partition the daughter cells secrete a new wall. Brand (1908) has recently confirmed these observations of cytokinesis in *Cladophora*. By treating the filaments with acetic and sulphuric acids he has distinguished several layers in the wall. The entire filament is surrounded by a continuous gelatinous sheath, within which lies the cell wall, which in turn is divided into an inner and outer layer, the inner layer being in contact with the plasma membrane. At cytokinesis the protoplast is pushed

back slightly from the wall at the point where division is to take place due to the gelatinization of the wall at that point. From the surface of the inner layer a ring-like cross wall develops and is deposited centripetally thus dividing the protoplast. The ring-like wall develops from within the inner wall layer so that the latter keeps pace with its growth and forms the end walls of the daughter cells. Such a stage as my figure 25 of *Protosiphon* is similar to the figures of Von Mohl and Brand of cytokinesis in *Cladophora*. The secretion of wall material into the furrow, continuous with the inner surface of the thallus wall is an essentially similar process in *Protosiphon* and *Cladophora* and in both cases takes place without relation to the position of and to the time of division of the nuclei. Lutman (1911) describes and figures cytokinesis in *Closterium* by the ingrowth of wall substance centripetally starting at the mother cell wall and extending into the constricting plasma membrane. Spindle fibers play no direct rôle in the process and a phragmoplast is entirely lacking. More recently McAllister (1931) in an account of the achromatic figure in *Spirogyra setiformis* reports that the new transverse wall between dividing cells develops centripetally through the activity of a phragmoplast which operates essentially as in the higher plants except for its centripetal development. Its centripetal development is undoubtedly the result of the formation of a large vacuole in between the two nuclei, so that the kinoplasm is pushed centrifugally nearer the wall. In *Spirogyra* cytokinesis is closely related to nuclear division in point of time as in the higher plants.

On the other hand coenocyst formation in *Protosiphon* is suggestive of cytokinesis in certain pollen mother cells as described by Farr (1916, 1918, 1922a, 1922b) and W. K. Farr (1920), in which cell division is accomplished by centripetal furrows which meet at the center of the pollen mother cell at a point equidistant from the four nuclei. The wall substance of the mother cell flows into the furrows so that the bounding membranes of the microspores are formed at least in part from the wall material of the mother cell. Castetter (1925) describes a similar method of cytokinesis in the pollen mother cells of *Melilotus* in which cytokinesis occurs by centripetally developing surface furrows and a series of vacuoles. The mother cell wall, which he claims is made up of callose, flows into the furrows forming the primary walls of the microspores, on the surface of which the microspore protoplasts secrete additional callose. The manner of coenocyst formation in *Protosiphon* is thus essentially similar to cytokinesis in other algae such as *Closterium* and *Cladophora* and to cytokinesis by furrowing in the pollen mother cells of the higher plants. It differs, however, from cytokinesis in *Closterium* and the pollen mother cells in that in these forms, cytokinesis is closely related to the nuclei and their time of division, and

in *Protosiphon* it is thus in this respect more similar to cell division in *Cladophora*.

Comparison of the method of zoosporogenesis in *Protosiphon* with sporogenesis in other algae and fungi reveals many striking similarities. With a view to bringing together some of the data on spore formation in the Thallophyta I have summarized in tables 1 and 2 the names of the species, types of cytokinesis and the names of the investigators. The data on the Ascomycetes were omitted in view of the rather uniform occurrence of free-cell formation in the ascus. Examination of table 1 shows the preponderance of evidence that the prevailing method of spore formation in the coenocytic fruiting bodies of Myxomycetes, Chytridiales, Zygomycetes and Saprolegniales is that of progressive cleavage by furrows, vacuoles or both. In view of the occurrence of alternate contraction and swelling of the cleavage segments in some organisms it is possible that this phenomenon is rather widespread, and that the accounts of simultaneous cleavage in certain species of *Synchytrium* and *Saprolegnia* may be explained by the rapidity with which the earlier stages of cleavage take place, and the corresponding readiness with which they may be overlooked.

From the data on the Chlorophyceae shown in table 2 one can distinguish several categories: the first class includes forms in which the cells are uninucleate throughout the life cycle and remain in that condition up to the time of reproduction. Zoospore and daughter colony formation in these forms is accomplished by a series of nuclear divisions and endogenous cell bipartitions so that the number of cells ultimately produced is a multiple of two. To this group belong such forms as *Chlorogonium*, *Chlamydomonas*, *Gonium* and *Eudorina* and probably all the Volvocales. A similar condition obtains in the Ulotrichales such as *Ulothrix*, *Stigeoclonium*, *Ulva* and *Monostroma*. The second category embraces those genera in which the cell is multinucleate either throughout the life cycle or during the reproductive stage. In these forms zoospore formation is accomplished by progressive cleavage into an indefinite number of segments which develop into the spores. Among such forms should be included *Characium*, *Chlorococcum*, *Hydrodictyon*, *Cladophora* and *Protosiphon*, and probably all the Siphonocladales and Siphonales. As West (1916) considers the coenocytic habit to have arisen secondarily from the more primitive uninucleate condition, it may not be illogical to consider bipartition as the earliest method of cell division. The prevailing method of spore formation in large multinucleate sporangia is by progressive cleavage by furrows or vacuoles.

The homologies of the protospore of such forms as *Synchytrium decipiens* are valuable at least in suggesting possible analogies among certain

TABLE 1

Sporogenesis in certain Thallophyta: Fungi

ORGANISM	BIPARTITION	PROGRESSIVE CLEAVAGE	"SIMULTANEOUS CLEAVAGE"	AUTHORITY	DATE
MYXOMYCETES					
Trichia			" (?)	Strasburger	1884
Fuligo		"		Harper	1900
Ceratiomyxa		"		Olive	1907
Didymium		"		Harper	1914
Physarella		"		Bisby	1914
Stemonitis		"		Bisby	1914
Lycogala		"		Vonwiller	1919
Physarum		"		Howard	1931
CHYTRIDIALES					
Polyphagus		"		Wager	1913
Olpidium			"	Kusano	1912
Olpidiopsis			"	Barrett	1912
SYNCHYTRIUM					
S. Taraxaci			"	Dangeard	1899
S. Taraxaci		"		Harper	1899
S. decipiens		"		Harper	1899
S. Puerariae		"	"	Kusano	1909
S. endobioticum			"	Percival	1910
S. endobioticum			"	Curtis	1921
S. fulgens			"	Kusano	1930
ZYGOMYCETES					
Pilobolus		"		Harper	1899
Sporodinia		"		Harper	1899
Rhizopus		"		Swingle	1903
Phycomyces		"		Swingle	1903
Pilobolus		"		Schwarze	1922
Sporodinia		"		Schwarze	1922
Rhizopus		"		Schwarze	1922
Mucor (2 sp.)		"		Schwarze	1922
Circinella		"		Schwarze	1922
OOMYCETES					
Saprolegnia			" (?)	Rothert	1892
Saprolegnia		"		Schwarze	1922
Achlya		"		Schwarze	1922
Plasmopara			" (?)	Gregory	1912
Plasmopara		" (z)		Nishimura	1926
Phytophthora			" (?)	Ward	1887

of the organisms listed in the table. As Harper (1899) has pointed out, the protospore of *Synchytrium decipiens* represents the culmination of the cleavage process, and the further nuclear and cell division which follow belong to the category of embryonic growth. The homologue of the *Syn-*

TABLE 2

Sporogenesis in certain Thallophyta: Chlorophyceae

ORGANISM	BIPARTITION	PROGRESSIVE CLEAVAGE	"SIMULTANEOUS CLEAVAGE"	AUTHORITY	DATE
Chlamydomonas	" (z)			Kater	1929
Chlorogonium	" (z)			Hartmann	1919
Gonium	" (d.c.)			Hartmann	1924
Eudorina	" (d.c.)			Hartmann	1924
Volvox	" (d.c.)			Harper	1918a
Chlorococcum					
C. infusionum			" (z)	Famintzin	1871
C. infusionum	" (z)			Artari	1892
C. infusionum		" (z)		Bold	1931
C. humicola	" (z)		" (a)	Bristol	1919
Chlorochytrium					
C. Lemnae		" (z)		Cohn	1875
C. Lemnae	" (z)			Klebs	1881
C. bienne	" (z)			Bristol	1920
C. paradoxum	" (z)			Bristol	1920
C. grande	" (z)		" (a)	Bristol	1917
Pediastrum		" (z)		Smith	1916b
Pediastrum	" (z)			Harper	1918b
Characium		" (z)		Smith	1916a
Tetraedron		" (a)		Smith	1918
Scenedesmus	" (d.c.)			Smith	1914
Sorastrum			" (z)	Geitler	1924
Hydrodictyon		" (z)		Timberlake	1902
Hydrodictyon		" (z)		Harper	1908
Ulothrix	" (z)			Gross	1930
Ulothrix	" (z)			Cholnoky	1932
Stigeoclonium	" (z)			Cholnoky	1928
Monostroma	" (z)			Carter	1926
Ulva	" (z)			Carter	1926
Cladophora		" (z)		Czempyrek	1930
Sphaeroplea		" (o)		Klebahn	1899
Codium			" (g)?	Williams	1925
Derbesia		" (z)		Davis	1908
Rhodochytrium		"	"	Griggs	1912

Letters in parentheses denote type of spore formed: (a) = aplanospore, (d.c.) = daughter colony, (g) = gametes, (o) = oospores, (z) = zoospores.

chytrium protospore in the developing zoosporangium of *Protosiphon*, is the uninucleate segment which is delimited at the completion of cleavage. It is homologous not only in method of formation but also in its subsequent development into swarmspores. The essential point of divergence is that in *Synchytrium decipiens* the multinucleate stage of the protospore, the spore which results from its embryonic development, is not immediately subdivided into swarmspores as is the case in *Protosiphon;* swarmspore formation in *Synchytrium* may be regarded as a germination stage as in certain Oomycetes. The formation of a clear area in the cytoplasm of the protospores between the nuclei before the final bipartitions is highly suggestive of what occurs in the final cleavage stages in *Fuligo*.

Harper has already pointed out the abbreviation of the cleavage process in *S. Taraxaci* as compared with *S. decipiens*, and in *Sporodinia* as compared with *Pilobolus*. The same relation holds in zoospore formation in the thalli as compared with the mature coenocysts of *Protosiphon* and the thalli of *Chlorococcum infusionum*, for although the process is essentially similar in all three, it is more abbreviated in the smaller coenocysts and *Chlorococcum* cells, in which because of their smaller size, the primary cleavage furrows may directly delimit uninucleate protospores.

In none of the other Chlorophyceae so far reported are there any stages homologous with the embryonic development of the protospore of *Protosiphon*. Timberlake describes the direct transformation of the uninucleate segment or protospore of *Hydrodictyon* into the swarmspore. This is true of the majority of forms reported. In view of Smith's (1930a) contention that *Halicystis* and *Protosiphon* are closely related it will be especially significant to compare the progressive cleavage in *Halicystis*, which he has described in a preliminary account, with the same process in *Protosiphon*, when figures of the former genus are available.

The mechanical principles involved and their operation in the process of cleavage are still uncertain. The similarity to segmentation of drying colloidal substances suggested to Harper that cleavage in the fungi which he studied might be essentially a drying-out process in which the nuclei function as centers of water retention. Cleavage in *Protosiphon*, *Hydrodictyon*[3] and the water molds, however, cannot be due to drying out in the same sense, since it occurs in these forms only when they are submerged. There can be no doubt from the data on the large number of forms investigated that changes from higher to lower water content of the spore plasm take place during zoospore formation. Loss of water in submerged forms, however, is plainly initiated and controlled throughout by the cell.

[3] See Harper (1908) for a discussion of cleavage in the living cells.

The hypothesis of water loss by drying as a possible explanation of the phenomenon of cleavage in submerged sporangia can possibly be applied to these forms if one emphasizes that this "drying" is autonomous on the part of the protoplasm.

It is apparent in the literature on sporogenesis outlined above, that some confusion exists as to the usage and meaning of the various terms describing types of cytokinesis. I have attempted in the following discussion, to clarify the different types of cell division described in the literature as far as I have been able to understand them.

According to Strasburger (1880) Schleiden first employed the term "free-cell formation" to designate the origin of cells within a protoplasmic mass, which process he considered to take place through the formation of granules in the homogeneous plasm (cytoblastema), about which other granular material collected, thus forming transparent vesicles, (cells) or "cytoblasts." The term was later extended by Hofmeister (1867) to include other cases of cell formation. First, the formation of the spores of the phanerogams, gymnosperms, vascular cryptogams and mosses; second, the formation of endosperm in the phanerogams, and the gametophyte from the megaspores of certain lycopods; and finally the development of the spores of lichens and ascomycetes, and those fungi which form their reproductive cells free within the mother cell. Strasburger (1880) in his general discussion of cell division and formation, concludes that the category of free-cell formation includes not only such cases as endosperm formation in the higher plants, but also such as the formation of the swarmspore in *Oedogonium* ("Vollzellbildung") and also the formation of a number of spores in a sporangium ("Vielzellbildung"). Probably on the basis of these conceptions, West (1916), Cohn (1875) and others refer to the formation of algal zoospores by "free-cell formation." Since 1880 however the term has come to be used in a rather limited sense, as referring especially to spore formation in the ascus, and Harper (1899) proposed to restrict its usage to the type of division found in the ascus. At the same time he pointed out that the process is quite different both from endosperm formation in the flowering plants and from the progressive cleavage by constriction which occurs in coenocytic sporangia; for in the ascus, a cell is delimited *within* the *protoplasm* of its mother, and a residual *epiplasm* remains. It is now generally agreed that the intersporal slime in coenocytic sporangia bears no relation to the *protoplasmic* residue in the ascus. In view of these confusing usages, and the fact, as pointed out above, that most cases of spore formation in algae and fungi occur by surface constrictions (bipartition and multipartition or progressive cleavage), it would perhaps make for greater clarity to retain the term "free-cell for-

mation" for those cases in which a cell is cut out from the protoplasm of a mother cell in such a way that residual protoplasm (epiplasm) remains. This category would then include spore formation in the ascus (which is accomplished through the agency of kinoplasmic fibers) as well as cell formation in the pro-embryo of *Ephedra* (Strasburger, 1880).

The term progressive cleavage was first applied by Harper (1899) to the process of segmentation of coenocytic fungus sporanges, in which delimitation of the spores is a progressive process, always progressive as far as the formation and deepening of the furrows is concerned, and often but not always progressive in that at first larger, and subsequently smaller masses of protoplasm are delimited.

Strasburger (1875) was probably the first to call spore formation simultaneous as Schwarze has pointed out, and the term has been applied to sporogenesis in a great series of forms by a number of investigators; as noted above, in view of the alternate swellings and contractions in sporangia and the rapidity with which sporogenesis is accomplished, it is reasonable to suppose on the basis of available data that there is no convincing evidence for the occurrence of a "simultaneous cleavage" process. If it could be demonstrated for some organism, that clefts are formed simultaneously throughout their entire extent such a process would have to be made a separate category. In my opinion no one has demonstrated it convincingly, and all cases of so-called "simultaneous cleavage" upon reinvestigation will undoubtedly fall into either the category of progressive cleavage or successive bipartition.

I have summarized these possible categories of cell as contrasted with nuclear division in the following table. As is the case with all such attempts, future investigation may show such a grouping false and misleading in many particulars.

Categories of cytokinesis as contrasted with karyokinesis

I. Segmentation by surface furrows or apparent constrictions including furrows originating at the surface of vacuoles.
   A. Bipartition: formation of two cells from one; cells not necessarily equal in size.
      1. Bipartition more or less directly following nuclear division.
         a. division of flagellates.
         b. holoblastic cleavage in homolecithal animal eggs.
         c. cell division in *Closterium*.
         d. zoospore formation in *Ulothrix*.
         e. conidia formation in the mildews.
      2. Bipartition not directly related in time and sequence to nuclear division.
         a. formation of the coenogametes in *Sporodinia*.
         b. zoospore formation in *Pediastrum*.
         c. cell division in *Cladophora*.

B. Multipartition, or progressive cleavage, including quadri-partition in certain pollen mother cells;[4] segmentation process relatively independent of nuclear division.
   1. Partition by surface furrows only.
      a. spore formation in the sporangia of many coenocytes such as *Synchytrium*, *Protosiphon*, etc.
      b. cleavage in centrolecithal animal eggs.
      c. segmentation of certain pollen mother cells (see Farr).
      d. coenocyst formation in *Protosiphon*.
   2. Partition by surface furrows and vacuolar furrows.
      a. columella formation in *Pilobolus* and *Sporodinia*.
      b. spore formation in *Rhizopus* and *Phycomyces*.
      c. segmentation of the pollen mother cells of *Melilotus* (see Castetter).
      d. cell formation in the plasmodium of human embryonic epithelium. (see Sharp, 1926, p. 15.)

II. Segmentation by cell-plate formation.
   A. Cell-plate formation closely associated with or involving the achromatic figure or kinoplasm. (Cytokinesis by phragmoplast.)[5]
      1. Path of cell plate a flat plane.
         a. Path centrifugal.
            Bryophytes, Pteridophytes, Spermatophytes—all vegetative cells; endosperm formation in the angiosperms (so far as known).
         b. Path centripetal.
            Spirogyra setiformis. (McAllister.)
      2. Path of cell-plate curved or circular.
         a. wall formation in the generative cells of *Abies* and *Picea* by curved phragmoplast[6] and cell-plate. (Hutchinson, 1914, 1915.)
         b. wall formation in the antheridium of certain ferns. (Strasburger, 1880.)
   B. Cell-plate formation apparently not involving directly the achromatic figure or kinoplasm.
      1. cell division in *Cladophora*.
      2. cell division in *Stypocaulon*. (Swingle, 1897.)
      3. segmentation of the oogonium in *Fucus*. (Farmer and Williams, 1898.)

III. Free-cell formation: cells arising within the protoplasm of a mother cell, leaving residual protoplasm: epiplasm in the ascus, periplasm in the oogonium of the Peronosporales.
   A. Free-cell formation clearly involving the activity of kinoplasmic fibers.
      1. spore formation in the ascus.
      2. cell formation in the pro-embryo of *Ephedra*.

[4] In preparations showing the divisions of the pollen mother cells in certain *Viola* hybrids, the author has observed the formation of as many as twelve uninucleate microspores by furrowing, which suggests a rather close connection between the process in pollen mother cells and other cases of furrowing by surface constriction.

[5] As pointed out to the author by Professor F. McAllister, the term *phragmoplast* was probably first used by Errera (1888).

[6] The activity of curved and circular phragmoplasts suggests in some degree, the process of free-cell formation as it occurs in the ascus.

B. Free-cell formation not directly involving the activity of kinoplasmic fibers (so far as known).
   1. cell-wall formation in the angiosperm zygote.
   2. formation of the female gamete in the Peronosporales.

I have listed under these possible categories, cases well known from the literature as far as I have been able to interpret them. A glance at the examples mentioned above, is sufficient to show that in all cases, the active agent of cytokinesis is either the constricting surface membrane, or kinoplasmic fibers. In view of the evidence for the close relation between kinoplasmic fibers and surface membranes, on the basis of future work it may be possible to clarify the relations between the categories still further.

The periodicity of swarmspore liberation so often reported in aquatic organisms like *Hydrodictyon*, *Pediastrum* and *Chlorococcum* is entirely absent from the terrestrial *Protosiphon* as far as my observations go. The stimulus which causes zoospore formation and escape in this organism is water, and submersion of mature thalli in water always results in liberation of motile cells without relation to time of day or light intensity.

The structure of the zoospore is similar to that of most of the Chlorophyceae so far studied cytologically. Although I have been unable conclusively to trace the origin of the blepharoplast from the nucleus, it is very certain that it is not a localized swelling of the plasma membrane as Strasburger described in the swarmspore of *Cladophora*. It always stains intensely with safranin, while the plasma membrane shows avidity for gentian violet; the presence of a rhizoplast is further evidence of its relation to the nucleus.

Should the reduction division be found to take place in the first division of the germinating zygospore, as is suggested by some cytological evidence at hand, *Protosiphon* would fall into the same category with the other haplobiontic Chlorophyceae such as *Coleochaete*. While gametes from the same plant do not usually conjugate, the occasional presence of zygospores within the old wall of a single plant would suggest that heterothallism is not so firmly established in *Protosiphon* as it has been reported to be in other Chlorophyceae such as *Cladophora*, *Enteromorpha*, *Chaetomorpha* and *Ulothrix*.

In studying ontogeny, structure and phylogeny of *Protosiphon* in relation to other members of the Chlorophyceae two views are to be considered, as Smith (1930a) has pointed out. First, *Protosiphon* may be looked upon as having developed from an organism of the *Chlorococcum*, *Chlorochytrium* type, in which the multinucleate condition became fixed, and the stimulus of a terrestrial habitat led to the formation of a subterranean rhizoid. The isogamous reproduction of *Protosiphon* is similar

to that in these genera. According to the second view, the coenocytic character of the thallus, and its differentiation into a rhizoidal and expanded aerial portion, are highly suggestive of conditions found among the Siphonales. On this basis *Protosiphon* could be included among those Siphonales with simpler vegetative structure and more primitive reproduction. It appears that the second view is more logical for several reasons. While the tendency to the coenocytic habit appears in certain forms of the Chlorococcales it has become the general rule in *Protosiphon*. The coenocytic habit according to most systems of classification is the most fundamental requisite for admission into the Siphonalian alliance.

The objection to including *Protosiphon* among the Siphonales because of its somewhat specialized habitat and form does not carry weight, in view of the fact that organs of anchorage appear again and again not only in terrestrial forms but in many members of the submerged Siphonales and even in such a form as *Spirogyra*, under certain conditions. On the contrary the somewhat special organization of the *Protosiphon* thallus is more in line with that of the Siphonales in general, since in this group the organization of the thallus reaches the highest state of development in the Chlorophyceae. Although no cases of isogamous reproduction have been reported among the Siphonales as far as I am aware, our present system of classification of Chlorophyceae is based largely on characters of the thallus, and there are many examples of the fact that degree of specialization in reproduction is not always correlated with a corresponding degree of thallus development. For these reasons isogamous reproduction in *Protosiphon* should be no serious obstacle to placing it among the Siphonales. Smith (1930 a) has noted that morphologically *Protosiphon* is the fresh water equivalent of the marine *Halicystis* except that the latter is considerably larger and has anisogamous gametes. For these reasons it seems more natural to consider the family Protosiphonaceae with the genera *Protosiphon* and *Halicystis* among the simpler Siphonales as West (1916) and Setchell and Gardner (1920) have done. One further point is significant. Among the Heterokontae if we accept West and Fritsch's (1927) and Pascher's (1925) group Heterosiphonales as exemplified by *Botrydium* alone, as an order parallel with the Siphonales of the Isokontae, it is at the very least highly inconsistent to relegate *Protosiphon* to the Chlorococcales.

SUMMARY

1. *Protosiphon botryoides* Klebs is a cosmopolitan species occurring on damp soil together with *Botrydium*. At the mature stage of vegetative development the thallus consists of a single cell with an aerial, expanded, bulb-like portion and an unbranched subterranean rhizoid. The plant is

coenocytic throughout its life cycle, returning to the uninucleate condition only during reproduction.

2. There is a large central vacuole and there is no evidence for the presence of distinct and localized chromatophores in the cells of *Protosiphon* such as are found in *Nitella*, *Botrydium* and other forms. On the contrary the chlorophyll completely pervades the cytoplasm, as evidenced by the random position of pyrenoids throughout the primordial utricle. Transition from the chlorophyll-bearing to colorless cytoplasm in the rhizoidal portion is gradual rather than abrupt.

3. The relation of the pyrenoid to starch formation is identical with that described by Timberlake in *Hydrodictyon*. Proteinaceous segments split off from the pyrenoid, are pushed out into the cytoplasm and become transformed into starch grains, staining successively brilliant red, reddish purple, and finally blue with the Fleming triple stain.

4. Reproduction is accomplished by separation of lobes and branches of thalli from young parent plants by septa; by formation of zoospores from thalli and coenocysts, which may function as gametes, fusing in pairs and forming thick-walled zygospores. The zygospore germinates after a period of dormancy into a new thallus. Zoospores also develop without fusion into new thalli. Zoospores which are not liberated from the parent cell develop walls within it and by their further growth into new thalli, rupture the mother thallus wall. Segmentation of the thallus protoplast into a number of multinucleate coenocysts occurs in periods of drought, and after continued exposure to sunlight these cysts develop a red pigment. In a moderately moist substratum the cysts can develop directly into the thallus, or when submerged in water, form zoospores. (Text fig. 1 illustrates the life history of *Protosiphon*.)

5. Coenocysts arise by the centripetal growth of furrows originating at the plasma membrane, and progressing through the cytoplasm. Wall material, continuous with the wall of the thallus, is secreted into the furrows by the protoplasm. The process is in some respects similar to that of cell division in *Closterium* and *Cladophora* and to cytokinesis in the pollen mother cells of certain higher plants.

6. Zoospore formation in thalli and cysts takes place by a process of progressive cleavage. The protoplast shrinks from the cell wall by extrusion of water from the plasma membrane and central vacuole. Furrows form at the surface of the plasma and tonoplast and cut progressively deeper into the protoplasm thus delimiting first multinucleate, and finally uninucleate segments or protospores. By further nuclear and cell division these segments give rise to the zoospores. The protospore of *Protosiphon* is similar to that of *Synchytrium decipiens*, *Pilobolus* and *Hydrodictyon*.

It differs from that in the last named organism only in undergoing embryonic development antecedent to forming the zoospores. The autonomous nature of dehydration of the protoplast during cleavage of submerged is stressed.

7. Zoospores and functional gametes of *Protosiphon* are bi-flagellate, naked protoplasts with a single stigma, two contractile vacuoles and with or without pyrenoids. The presence or absence of the pyrenoid is correlated with its disappearance or fragmentation during zoosporogenesis. The nucleus is anterior and connected to the blepharoplast by a single rhizoplast. In conjugation two gametes establish contact in the region of the blepharoplast and fusion proceeds from the anterior toward the posterior region. Syngamy occurs from three to four hours after the initiation of plasmogamy. As zoospores and zygospores come to rest the flagella are withdrawn into the cell. Gametes from the same thallus or coenocyst as a rule are incompatible, although occasionally zygospores may be found within a single mother cell.

8. Nuclear division is simultaneous throughout the thallus, but not completely synchronous, nuclei at the apex or base of the cell usually being more advanced. The structure of the nucleus is similar to that of the nuclei of the higher plants in that nucleoli, chromatin and nuclear membranes are present. The nucleole apparently plays no part in the formation of the chromosomes, disappears during the prophases, and is reorganized in the daughter nuclei.

9. *Protosiphon* along with *Halicystis* should be placed among the simpler Siphonales as West (1916) and Setchell and Gardner (1920) have done, rather than retained among the Chlorococcales.

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Explanation of plates 10–19

All figures were drawn with the aid of a camera lucida, using a Leitz 1/16 oil immersion objective or a Bausch and Lomb 1.8 mm Fluorite oil immersion objective with various compensating oculars. The approximate magnification is given in the descriptions of the figures. Plates 13 and 14 are slightly reduced.

Fig. 1. Longitudinal section of an almost mature thallus. ×760.

Figs. 2, 3. Median longitudinal sections of rhizoids of young thalli. ×1200.

Fig. 4. Immature thallus from densely inoculated culture. ×1540.

Fig. 5. Cross section of thallus through expanded portion; tonoplast separated from primordial utricle in certain regions. ×760.

Fig. 5a. Portion of preceding thallus enlarged. ×2400.

Figs. 6–10. Starch formation from pyrenoids. ×2400.

Figs. 11, 12. Condition of resting nuclei. ×2400.

Figs. 13, 14. Prophases of nuclear division. ×2400.

Figs. 15–17. Anaphase, equatorial plate, and telophase stages. ×2400.

Fig. 18. Longitudinal section of an immature thallus illustrating progressive synchronism of nuclear division. ×1540.

Figs. 19–23. Stages in the development of the thallus from the zoospore stage. ×1540.

Fig. 24. Dormant zygote from same preparation as preceding. ×1540.

Figs. 25–27. Sections of encysting thalli at different stages. ×1540.

Fig. 28. Encystment completed; thallus completely divided into mature coenocysts. ×760.

Fig. 28a. Median longitudinal section of a rhizoid after encystment. ×1540.

Fig. 29. Cross section of mature thallus after immersion in water, illustrating progressive cleavage in zoosporogenesis; pyrenoid fragments evident in cytoplasm; nuclei in interphase condition. ×1540.

Fig. 30. Median longitudinal section of an immature thallus in the protospore stage of zoosporogenesis. Note remnant of the central vacuole. ×1540.

Fig. 31. Cross section of thallus illustrating primary segmentation by furrows into multinucleate blocks, some of which are undergoing further furrowing.

Fig. 32. Oblique section of thallus showing early stages of progressive cleavage and the vacuolar remnant at one side of the protoplast. ×1540.

Fig. 33. Portion of a section of a thallus at the uninucleate or protospore stage at the completion of cleavage. ×1540.

Fig. 34. Nuclear division in the protospores illustrating almost complete synchrony. ×1540.

Fig. 35. Bipartition of binucleate protospores after nuclear division. ×1540.

Fig. 36. Dividing protospores more highly magnified showing the clear zone in the equatorial region. ×2400.

Fig. 37. Partial section of thallus shortly after motility of zoospores has been initiated. ×1540.

Fig. 38. Immature coenocyst soon after formation with highly vacuolate protoplasm. ×1540.

Figs. 39, 40. Successive stages in the maturation of coenocysts. ×1540.

Fig. 41. Zoosporogenesis in an immature coenocyst. ×1540.

Fig. 42. Partial section of a more mature coenocyst during cleavage. ×1540.

Fig. 43. Cleavage in a small, but mature coenocyst. ×1540.

Fig. 44. Protospore stage in zoosporogenesis of a coenocyst. ×1540.

Fig. 45. Zoospores soon after initiation of motility within the mother thallus, greatly magnified; rhizoplast, blepharoplast and flagella visible. ×2400.

Fig. 46. A single zoospore initial before the development of flagella, illustrating the close association of the blepharoplast with the nucleus.

Fig. 47. Mature and actively swimming zoospores before liberation from the mother thallus. ×1540.

Fig. 48. Division of the protospore nuclei at the conclusion of cleavage in a coenocyst. ×1540.

Fig. 49. Protoplasts formed as a result of bi-partition of the protospores. ×1540.

Fig. 50. Bi-partition after nuclear division in the protospores. ×1540.

Fig. 51. Probable second nuclear division of the protospores. (Compare with Figs. 48 and 49.) ×1540.

Fig. 52. Zoospores at the initiation of motility within the mother coenocyst. ×1540.

Figs. 53–58. Stages of plasmogamy. ×2500.

Figs. 59–61. Stages of syngamy. ×2500.

Fig. 62. Dormant zygote. ×2500.

Figs. 63, 64. Prophases of nuclear division in the germination of the zygote. ×2500.

Fig. 65. Binucleate stage of the germinating zygote. ×2400.

Fig. 66. Tetranucleate stage of the germinating zygote. ×2500.

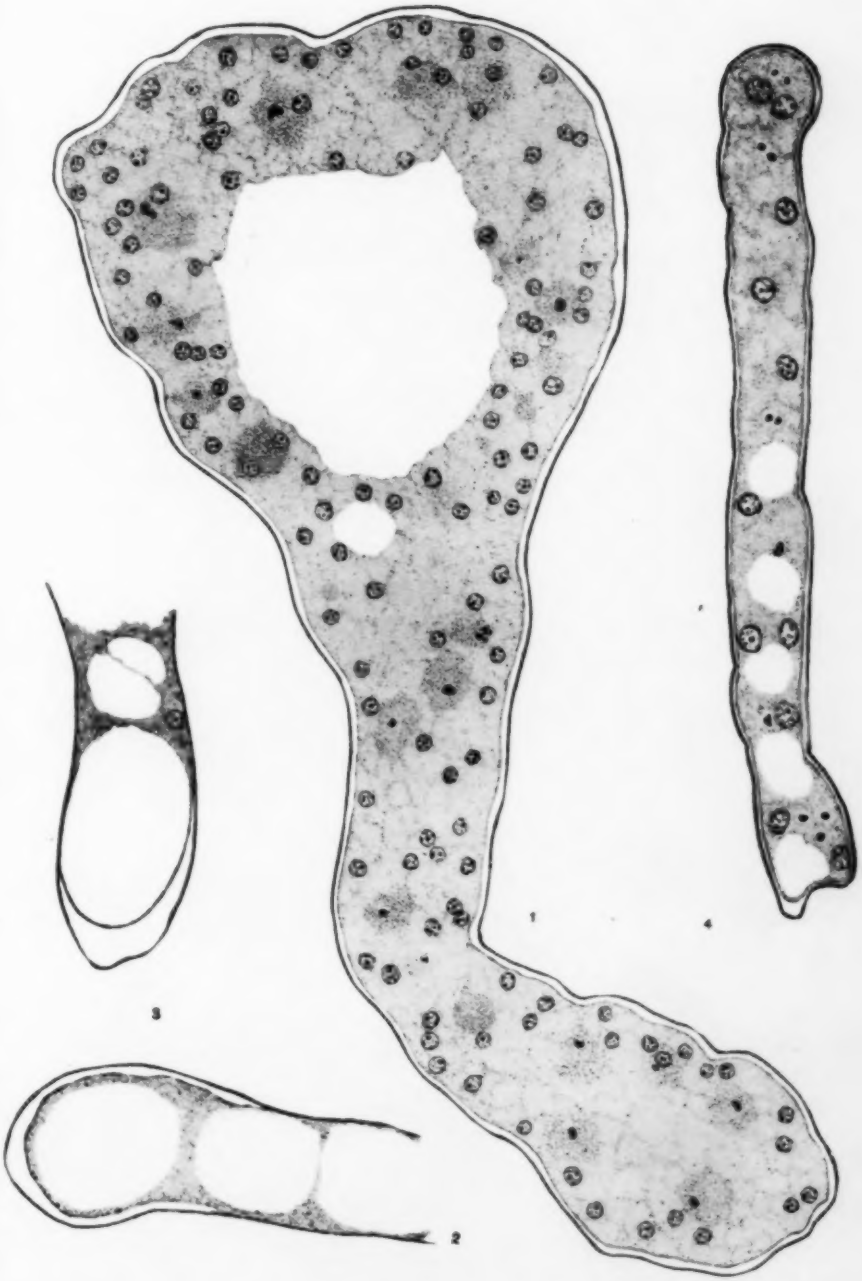
(Figs. 67–75 are from living cells.)

Fig. 67 a–h. Plasmogamy of living gametes in a hanging drop culture. Contractile vacuoles, stigmas and flagella visible. ×1540. i. Portion of the margin of the drop illustrating zoospores and zygotes as they come to rest. ×1540.

Fig. 68. Variation in size and shape of zoospores. End view illustrated at *a*. ×1540.

Figs. 69–73. Stages of growth of the zoospore into the thallus. 69a. zygote entering dormancy. ×1540.

Figs. 74, 75. Branching of young thalli and separation of the branches by partitions. ×1540.



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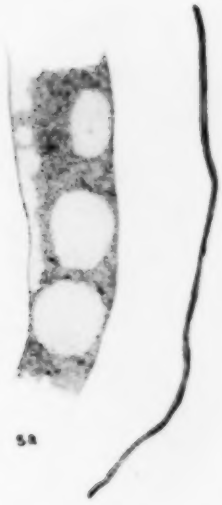

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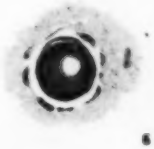

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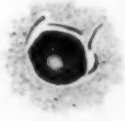

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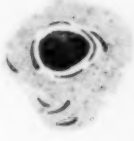

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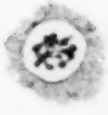

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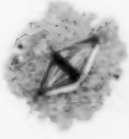

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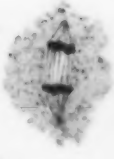

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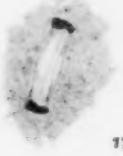

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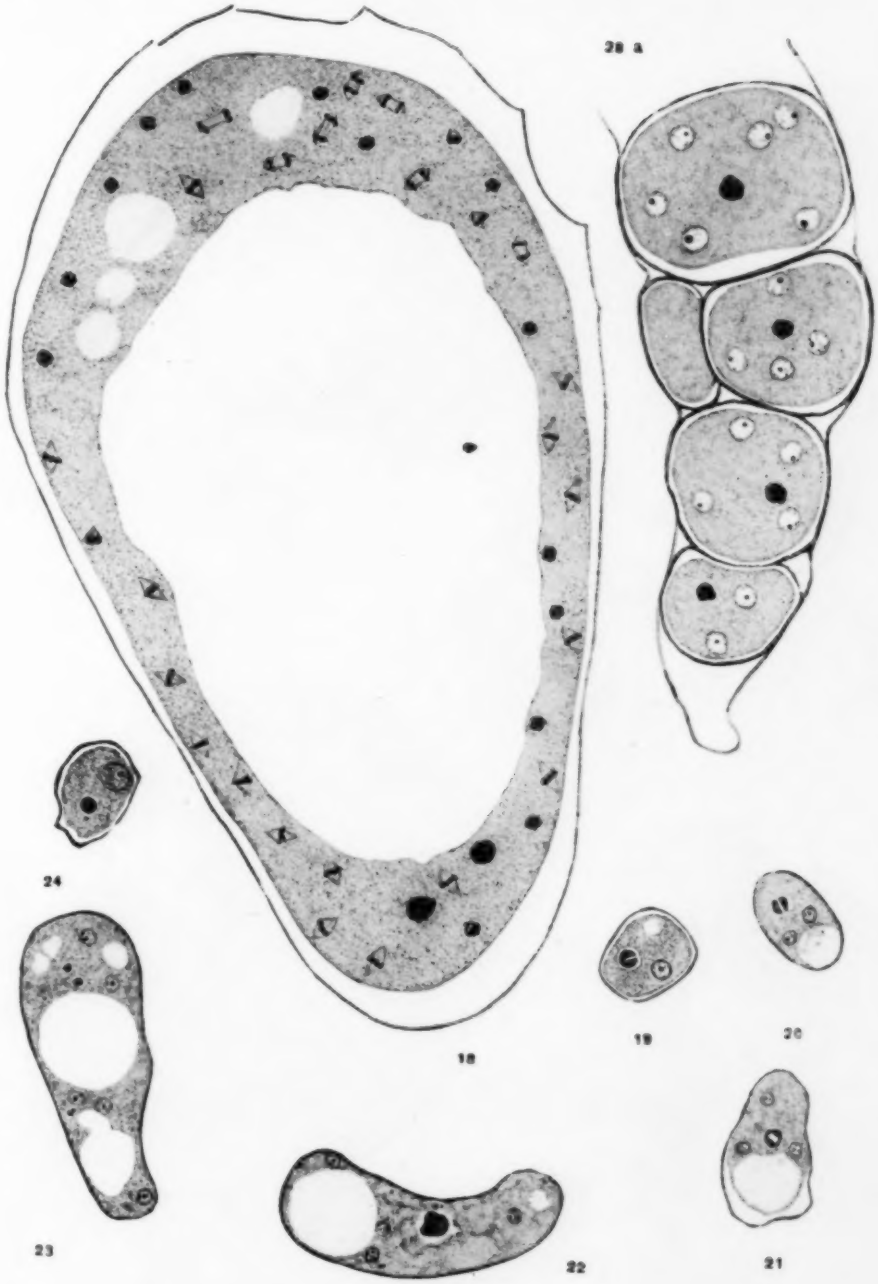

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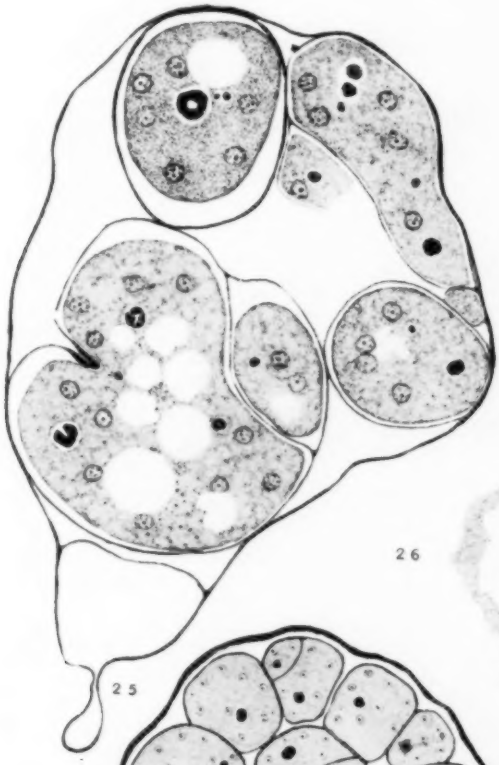

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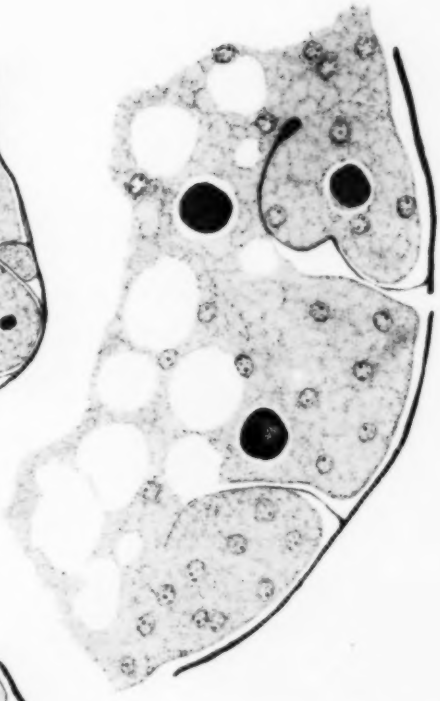

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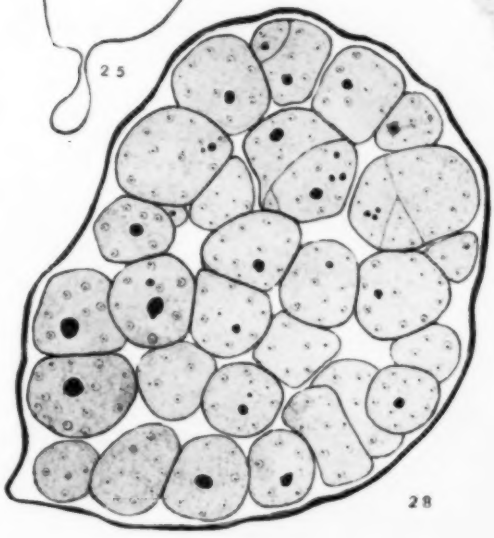

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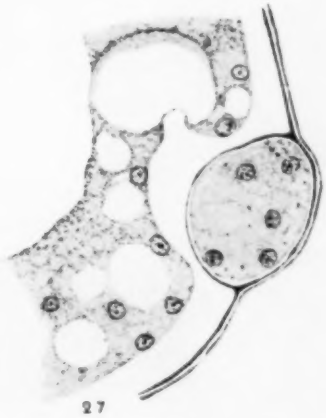

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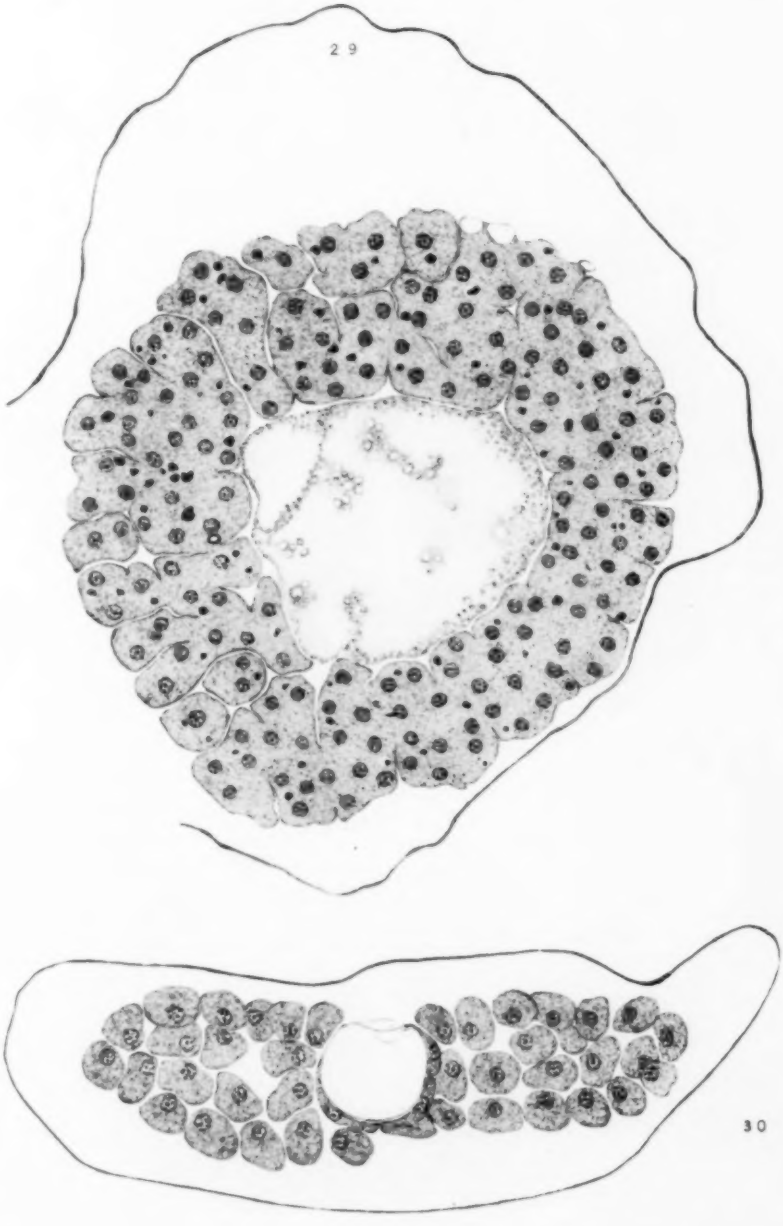

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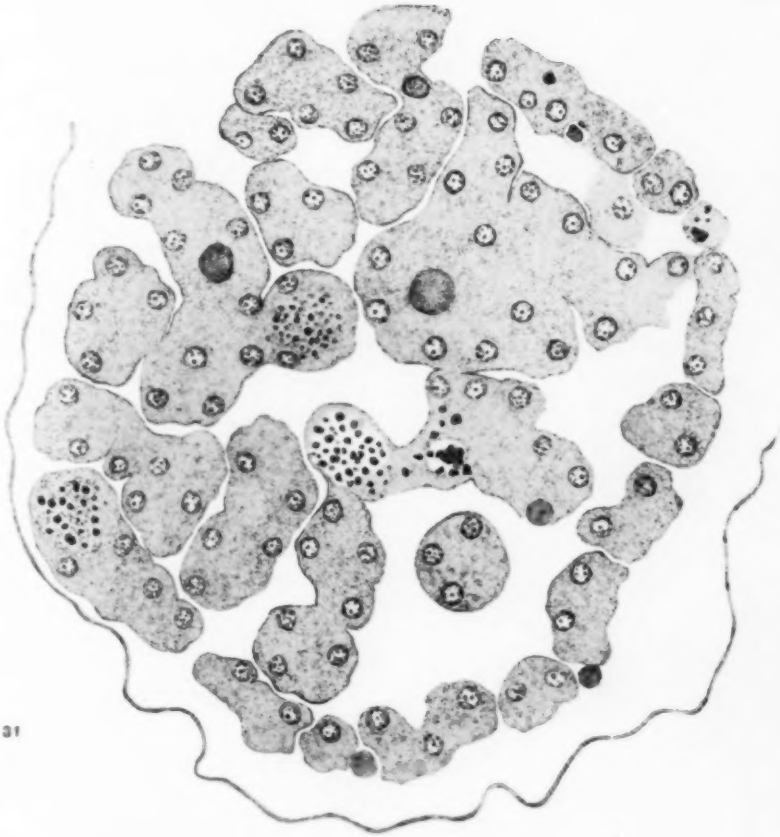

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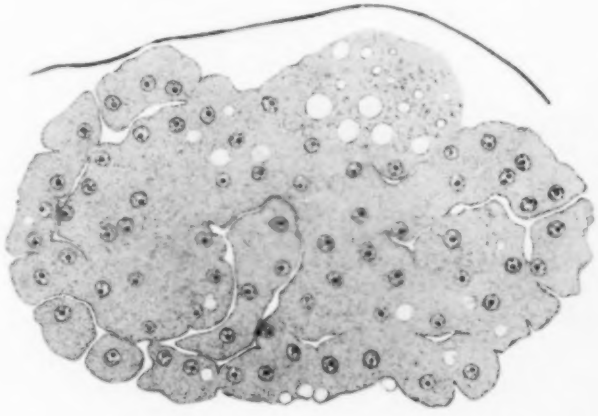

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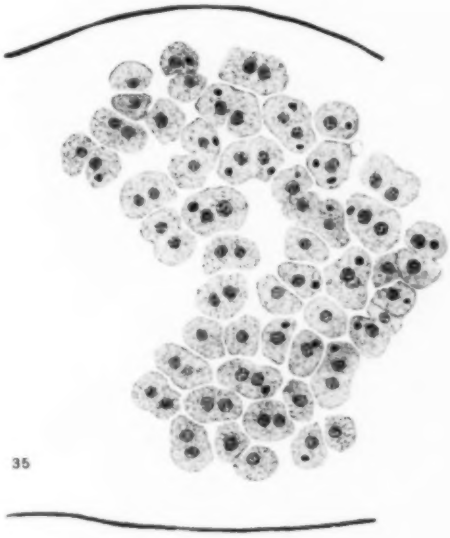

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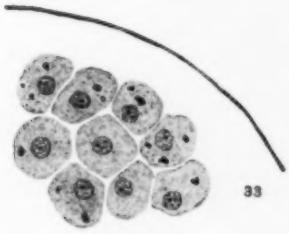

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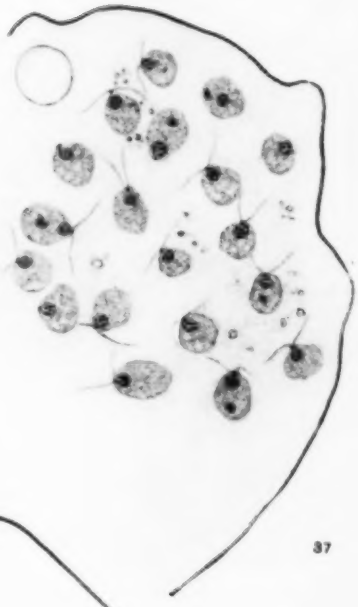

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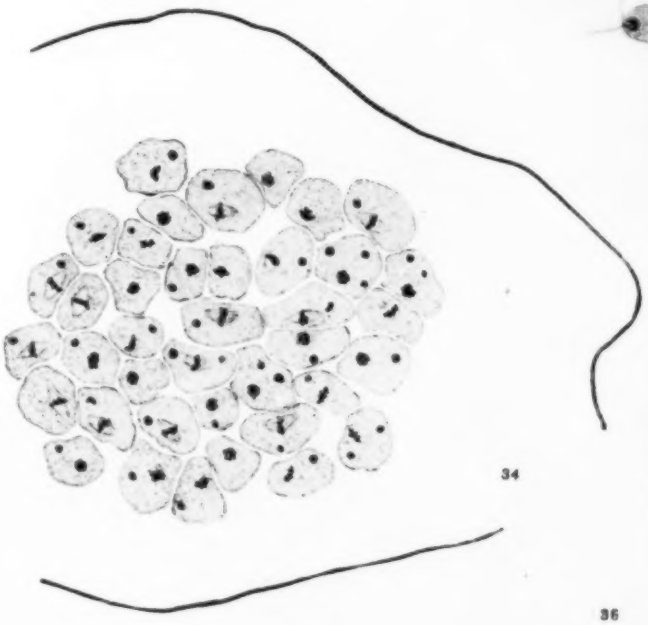

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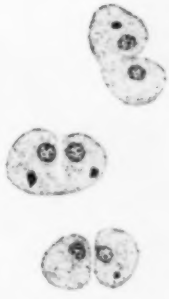

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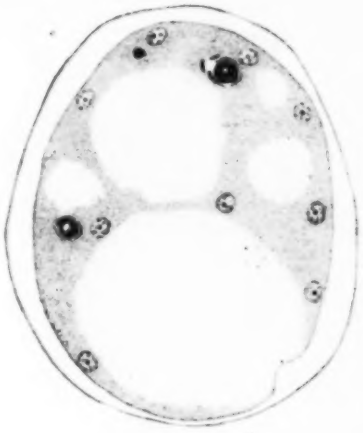

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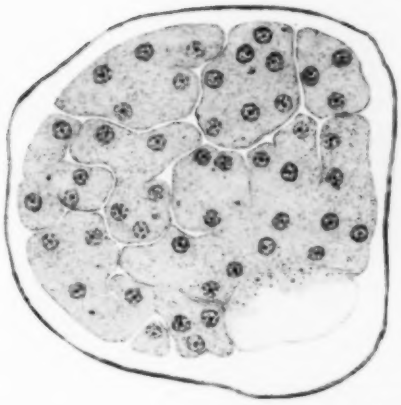

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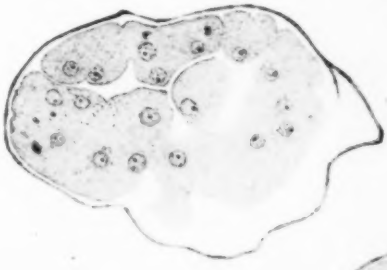

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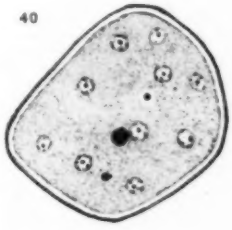

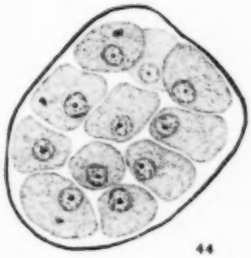

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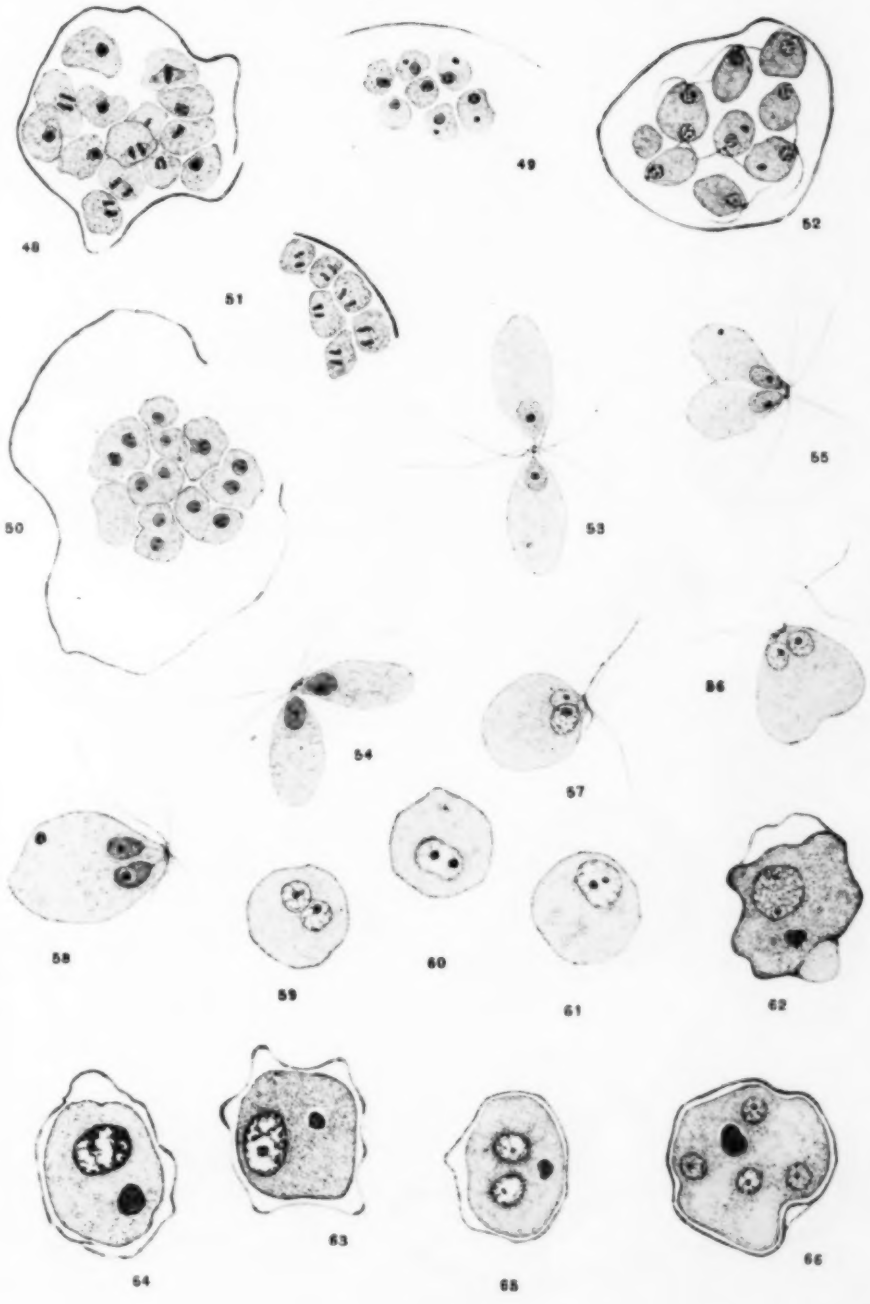

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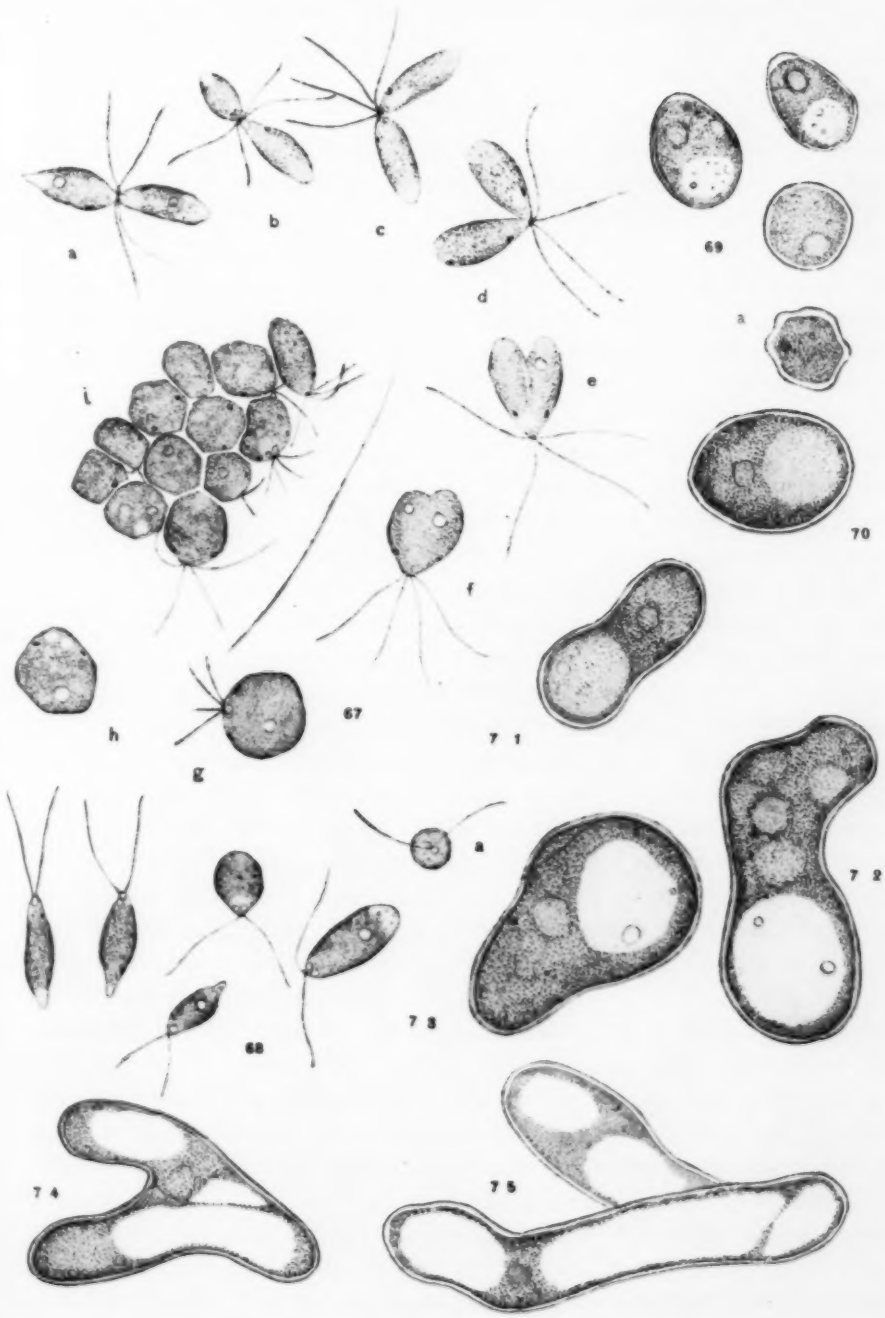

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Plant succession at the borders of a kettle-hole lake

H. W. Graham and L. K. Henry

(with eight text figures)

During the summer of 1926 while located at a camp at Deep Pond near Wading River, Long Island, the senior author was impressed with the striking zonation of the plants on the shore of the lake. These zones were not the associes commonly found at the borders of lakes representing increasingly drier conditions progressing from the water, for several zones occurred on the dry beach where the edaphic conditions were apparently more or less uniform. Deep Pond is a kettle-hole lake dependent upon the main water table, the surface of the lake being in the plane of the ground water. Consequently, fluctuations in the height of the water table cause variations in the level of the lake. It was learned from local residents that the level of the lake had been falling for several years and was, in 1926, at a very low stage. A study of the vegetation about Deep Pond at that time indicated that the zonal segregation of the shore plants was associated with the annual recessions of the lake during the preceding years.

The results of these observations were discussed with the junior author who was located at Deep Pond during the summers of 1929 and 1931. His investigations in those two years showed that the assumptions made in 1926 were largely correct, namely, that the vegetation on the shore of the lake is an ever-changing community responding to the fluctuations in environment due to the oscillations of the lake level. During periods of rising water there is a denudation of the area submerged caused by drowning and the scouring action of the waves and ice. During this phase of the cycle there is a progression of hydrophytic and aquatic plants from the floor of the lake. During periods of falling water level denuded strips of shore are exposed and there is a progression of mesophytic and xerophytic plants from the pine barrens bordering the lake.

During the winter months of a period of lowering water level, portions of the beach are exposed which cannot be invaded by plants because of the dormant state of the vegetation. Consequently, by the time growth is resumed in the spring a strip of denuded beach has been exposed. Certain moisture-loving plants quickly occupy this strip, the same plants which occupied the adjacent strip exposed the preceding year. This adjacent strip, however, is now occupied predominantly by other plants, more mesophytic in character, which were in the strip exposed two years before. As a consequence, this succession of plants in the denuded shore presents concentric zones about the lake which represent the annual recessions of

the lake level. As the lake recedes certain hydrophytes are left stranded above water. The more tolerant of these may remain for a time in the first shore zone and are sometimes found there with some of the more mesophytic forms. Similarly, during periods of rising water level, particularly

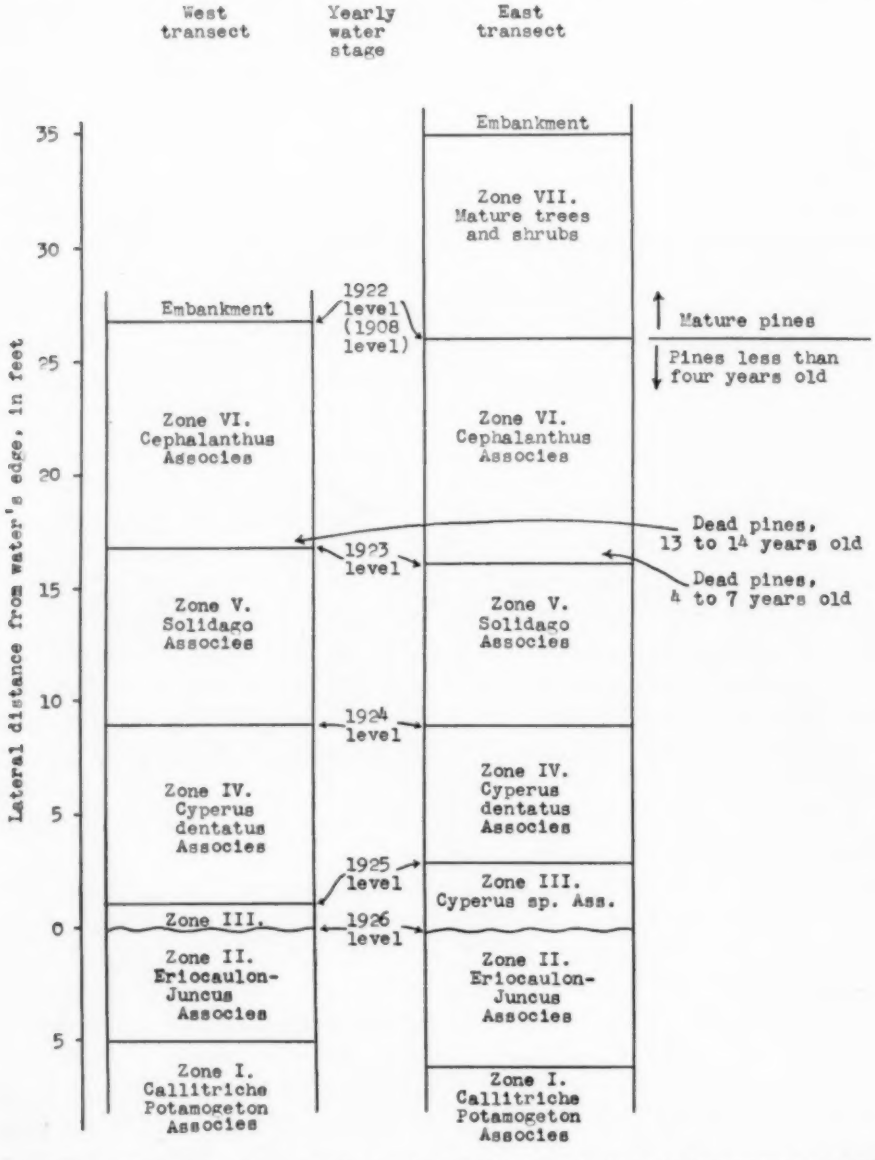

Fig. 1. Vegetation zones at the borders of the lake during the low water stage of 1926.

when the rise is rapid, certain submerged mesophytes may survive for a short time with the invading hydrophytes.

The lake level in 1926 was unusually low and proved to be the lowest of the current recession. When the lake was visited three years later, in 1929, its level had risen considerably, drowning many of the plants which had become established on the exposed beach during the recession ending in 1926. This rise which was observed in 1929 probably reached its maximum late in that year for local reports stated that the lake level was again dropping during 1930. This recession continued to 1931. When the lake was visited in 1931 it was found to have dropped 15 inches since 1929, ex-

Fig. 2. Vegetation on west shore of lake in 1926 at time of lowest water level of the recession. In 1922 the lake was up to the base of the embankment to the left. Numerals indicate the four shore zones extending from this level to the water's edge. Footpath can be seen in the center of Zone IV.

posing from 12 to 13 feet of beach line. On this exposed beach another plant succession was already taking place. The remains of many of the plants of the previous succession still persisted and were mixed with the plants of the latest succession.

Thus, from these few years' observations it was strikingly demonstrated that the shore of the lake extending from the pine barrens at its upper margin to the lowest level attained by the lake is the scene of an ever-changing plant community, an area which is being alternately denuded and reinhabited by a host of plants, the scene of a series of arrested

successions, each succession surviving for only a few years, depending upon the fluctuation in the level of the water table.

In August, 1926, the shore succession probably was as far advanced as it is ever permitted to proceed. At this time two belt transects were made through the zones to determine the qualitative nature of the zones or annual developments of the succession, one on the west side of the lake, the other on the east side. In each transect four zones on the beach were evident marking the annual recessions of the lake from the 1922 high to the 1926 low. Each of these zones was a different plant associes representing later stages in the succession progressing from the lake toward the pine barrens. In addition to these four seral units on the shore, there were two associes in the water. The first of these, the *Callitriche-Potamogeton* associes, occurred in deep water and probably varied little with fluctuations in lake level. The second, the *Eriocaulon-Juncus* associes, was in shallow water and probably migrated somewhat to different elevations in accordance with the variations in lake level. In addition there was one zone in the east transect which, because of the wider beach, was above the usual maximum elevation of the lake and, consequently, presented a much more advanced stage in the succession than the lower zones. It contained such mesic forms as *Vaccinium*, *Betula populifolia*, and *Pinus rigida*. These seven zones were numbered from I to VII beginning with the associes in the deep water. They are diagrammatically represented in Figure 1. Following is a list of the species found in each zone with some accompanying remarks. The nomenclature is according to Gray's Manual of Botany, seventh edition, and the specimens collected during the study are deposited in the Herbarium of the Carnegie Museum at Pittsburgh, Pa.

I. CALLITRICHE-POTAMOGETON ASSOCIES

Callitriche heterophylla Pursh. Potamogeton Robbinsii Oakes. Potamogeton hybridus Michx.

The first two species were established in the lake at depths of 14 to 15 feet below the surface. Both were commonly found washed ashore, probably loosened from the bottom by fish. They were seen and collected *in situ* only by divers. *P. hybridus* grew in five to six feet of water. This associes represents the initial stage in a hydrarch succession which will ultimately end in a *Pinus rigida* association after the lake has been completely filled by silting and the accumulation of plant remains. This succession cannot develop in the lake until it has been rendered shallow by these processes. However, at the edges of the lake the periodic lowering of the water level with resultant emergence of more or less denuded areas permits the suc-

cession to proceed for a limited time until a recurrence of high water destroys the plant community and initiates a new succession.

II. ERIOCAULON-JUNCUS ASSOCIES

Eriocaulon articulatum (Huds.) Morong
Juncus articulatus L.
Juncus dichotomus Ell.
Juncus pelocarpus Mey.

This zone represented the second stage in the hydrarch succession and occurred in shallow water. In 1926 at its upper limit the water was only a few inches deep. During periods of higher lake levels it was in deeper water. The members of this associes can adjust themselves to various water levels by varying the lengths of their stems.

Fig. 3. Drowning of the Solidago Associes on the west shore of high water in 1929.

III. CYPERUS ASSOCIES

Coreopsis rosea Nutt.
Cyperus sp.
Gratiola aurea Muhl.
Juncus articulatus L.
Polygonum Hydropiper L.

This zone which was the third stage in the hydrarch succession represented the first shore zone or the first stage in the secondary succession which was initiated on the denuded beach exposed in the spring of 1926.

It occurred in a very narrow band at the water's edge extending in the west transect only one foot up the shore and in the east transect about four feet from the water.

IV. CYPERUS DENTATUS ASSOCIES

Acalypha gracilens A. Gray
Ambrosia artemisiifolia L.
Coreopsis rosea Nutt.
Cyperus dentatus Torr.
Epilobium coloratum Muhl.
Erechtites hieracifolia (L.) Raf.
Erigeron canadensis L.
Gratiola aurea Muhl.
Juncus articulatus L.
Lactuca canadensis L.
Linaria canadensis (L.) Dumont
Lycopus sessilifolius A. Gray
Polygonum Hydropiper L.
Polygonum Persicaria L.
Quercus coccinea Muench. (seedlings)
Setaria glauca (L.) Beauv.
Stachys hyssopifolia Michx.
Taraxacum erythrospermum Andrz.

Fig. 4. Arresting of succession on the east shore in 1929. Inundation of *Solidago* Associes.

Coreopsis rosea Nutt. and *Stachys hyssopifolius* Michx. were subdominant in this associes. This associes represented the second shore zone and occupied the strip of beach exposed in the spring of 1925. It thus represented a stage in the succession one year in advance of the Cyperus Associes. Its position is shown in Figure 1.

V. SOLIDAGO ASSOCIES

Agrostis alba L.
Agrostis hyemalis (Walt.) B.S.P.
Andropogon virginicus L.
Coreopsis rosea Nutt.
Gerardia sp.
Gnaphalium polycephalum Michx.
Juncus effusus L. (moribund)
Lechea villosa Ell.
Linaria canadensis (L.) Dumont
Oenothera muricata L.
Panicum sp.
Salix discolor Muhl.
Salix nigra Marsh.
Solidago tenuifolia Pursh.
Stachys hyssopifolia Michx.
Strophostyles umbellata (Muhl.) Britton
Verbascum Thapsus L.
Verbena hastata L.

Coreopsis rosea Nutt. was subdominant in this zone. A society of *Strophostyles umbellata* (Muhl.) Britton occurred in one place on the west shore to the exclusion of everything else. This associes represented the third shore zone and occupied the strip of beach exposed in the spring of 1924.

VI. CEPHALANTHUS ASSOCIES

Andropogon virginicus L.
Apocynum cannabinum L.
Asclepias amplexicaulis Sm.
Baptisia tinctoria (L.) R. Br.
Betula populifolia Marsh.
Cephalanthus occidentalis L.
Chrysopsis falcata (Pursh.) Ell.
Chrysopsis mariana (L.) Nutt.
Cyperus filiculmis Vahl.
Deschampsia flexuosa (L.) Trin.
Eupatorium hyssopifolium L.
Helianthemum canadense (L.) Michx.
Hypericum perforatum L.
Juncus articulatus L.
Juncus dichotomus Ell.
Juniperus virginiana L.
Lyonia mariana (L.) D. Don.
Melampyrum lineare Lam.
Myrica carolinensis Mill.
Panicum Commonsianum Ashe.
Pinus rigida Mill.
Populus tremuloides Michx.
Quercus velutina Lam.
Robinia Pseudo-Acacia L.
Rubus villosus Ait.
Salix nigra Marsh.
Smilax glauca Walt.
Solidago odora Ait.
Solidago tenuifolia Pursh.
Verbena hastata L.

No species was really dominant in this zone in 1926 but, since some of the specimens of *Cephalanthus* were from ten to fifteen years old, it is probable that over long periods of time this species is the predominant member of the community. While other more mesophytic plants are killed by submergence, *Cephalanthus* probably often survives short periods under water. This area is probably never submerged for any extended time since it is at the level of the maximum elevation of the lake.

It is obvious that this was a more mature stage than any of the preceding as it contained several species of trees, some of which are characteristic of the climax formation of the region. It is highly significant that none of these trees was over four years old. This associes represented the fourth shore zone and occupied the strip of beach exposed in the spring of 1923 following the peak of the rise ending in 1922. The oldest of the above-mentioned trees apparently seeded in the early spring of that year. The upper limit of this zone marks the limit of the high water in 1922.

Fig. 5. Dead *Pinus rigida* on the west shore, 13 to 14 years old, probably established after the high water of 1908 and killed by the high stage in 1922. The living pines to the left were above the latter stage. Photo taken in 1926.

Another significant feature of this zone was a line of dead pitch pines in the lower part of the zone. Counts of growth rings showed that the dead trees in the west transect had died at the age of 13 to 14 years while those in the east transect had lived only 4 to 7 years. The significance of these trees is discussed below in connection with cyclic variation in lake level.

ZONE VII

This zone was apparently above the usual maximum rise of the lake for it showed an advanced stage in the succession. It comprised such mesic forms as birches, maples, oaks, and pines. This zone did not occur in the west transect because on the west side of the lake the steep embankment

bordering the lake extended to the high water line of 1922 or to Zone VI. Since Zone VII was above the level of usual maximum rise, it is of no particular interest in this study and will not be described in detail.

In a reconnaissance of Long Pond, another kettle-hole lake about one mile southwest of Deep Pond, vegetation zones similar to those at the latter lake were found. It is probable that the vegetation changes observed at Deep Pond are more or less characteristic of all the lakes in that general region.

SUCCESSION COMPLEX DURING RISING WATER AS OBSERVED IN 1929

The presence of the junior author at Deep Pond in 1929 and 1931, three and five years after the first studies were made, afforded an oppor-

Fig. 6. Dead *Pinus rigida* on the east shore 4 to 7 years old, probably established after the high water of 1915 and killed by the 1922 maximum. Photo taken in 1926.

tunity to obtain definite information regarding the effect of the changing lake level upon the vegetation bordering the lake. Following the minimum level of 1926 the lake rose so that by the summer of 1929 the shore line was submerged for a distance of several feet. During that summer the lake continued to rise. This inundation, of course, caused considerable change in the zonal nature of the shore vegetation. There seemed to have been little denudation of the submerged area, possibly because the water

rose rather rapidly and no area was long at the water's edge where the scouring action of the waves is the strongest. Some of the shore zones of 1926 could still be recognized.

Zone I, *Callitriche-Potamogeton* associes, was unchanged as it occurred in deep water and was unaffected by small changes in water level.

Zone II, *Eriocaulon-Juncus* associes, was also practically unaltered. It probably had adjusted itself and migrated somewhat in response to the higher water for it was found in about the same depth of water as in 1926.

Although no changes in floristic composition occurred in the aquatic zones, it was not so with the shore zones. The zones above water had advanced three years in the succession while the recently submerged areas presented a complex structure.

Zone III, *Cyperus* associes, could not be distinguished in 1929 nor could the species constituting it be found. This was a narrow zone at the water's edge in 1926 and probably was quickly submerged by the rising water.

Zone IV, *Cyperus dentatus* associes of 1926, was under water in 1929 and was the scene of opposing processes. This zone was probably represented in 1927 and 1928 by a community similar to the *Solidago* associes of 1926 for this is the next successional step after the *Cyperus dentatus* associes. However, the high water had arrested succession and initiated a new one during 1928 and 1929. Many of the plants of the *Solidago* associes were still thriving but some had disappeared and many more hydric forms had arrived. The following species characteristic of the *Solidago* associes of 1926 were not found in Zone IV in 1929:

Agrostis alba L.
Gerardia sp.
Gnaphalium polycephalum Michx.
Oenothera muricata L.
Verbascum Thapsus L.

The more hydric forms which occurred and which were not characteristic of the *Solidago* associes of 1926 were as follows:

Ambrosia artemisiifolia L.
Bidens frondosa L.
Cyperus dentatus Torr.
Erigeron canadensis (L.) Dumont
Eupatorium hyssopifolium L.
Gratiola aurea Muhl.
Hypericum canadense L.
Hypericum perforatum L.
Impatiens alba Nutt.
Juncus articulatus L.
Polygonum Hydropiper L.
Polygonum Persicaria L.
Viola lanceolata L.

A few of the latter group were new arrivals while others probably represented individuals remaining from 1926 when this was the *Cyperus dentatus* associes.

Zone V, the *Solidago* associes of 1926, had progressed to a stage com-

parable to the *Cephalanthus* associes. Although *C. occidentalis* L. had not migrated to this area, other species characteristic of that stage were present. It will not be necessary to list these. Species found in this zone in 1929 which did not occur anywhere on the shore in 1926 are:

Ambrosia artemisiifolia L.
Carex tribuloides Wahl.
Cassia nictitans L.
Hypericum canadense L.
Lactuca canadensis L.
Panicum columbianum Scribn.
Salix humilis Marsh.

In 1929 the rising lake had partially inundated this zone so that succession was halted in its lower portion. Of the species in this zone characteristic of the *Cephalanthus* associes of 1926 one, *Pinus rigida*, is of particular importance in connection with studies of periodicity in the fluctuation of the lake level discussed below. Small pines had become established and were growing to the water's edge.

Zone VI, the *Cephalanthus* associes of 1926, showed a more advanced state in 1929 by the addition of certain species and by the greater size of the trees and shrubs found in 1926. The additions included:

Acer rubrum L.
Anaphalis margaritacea (L.) B. & H.
Apocynum androsaemifolium L.
Cassia nictitans L.
Desmodium marilandicum (L.) DC.
Epilobium angustifolium L.
Lactuca canadensis L.
Lechea villosa Ell.
Leucothoë racemosa (L.) A. Gray
Lysimachia quadrifolia L.
Myrica asplenifolia L.
Panax quinqefolia L.
Prunus serotina Ehrh.
Pteris aquilina L.
Rhus copallina L.
Rubus villosus Ait.
Salix pentandra L.
Salix sericea Marsh.
Sassafras variifolium (Salsib.) Ktze.
Vaccinium vacillans Kalm.

Zone VII, which occurred above usual high waters and was in the east transect only, was essentially unchanged except for the greater age of its members.

OBSERVATIONS IN 1931

The peak of the rise in lake level was reached late in 1929 for the water was reported receding in the summer of 1930 and was observed doing so in 1931. The level of the lake in the summer of 1931 was 15 inches lower than in the summer of 1929; a strip of shore about 13 feet wide had been re-exposed. At the 1929 maximum the water had not risen to the point which appeared to be the level of the usual maximum rise, i.e., at the upper limit of Zone VI of 1926, but only into Zone V. Consequently, Zone VI and most of Zone V showed in 1931 a continuance of the succession ob-

served in 1926 and 1929 while on the shore below this level the old zones defined at the close of the recession in 1926 were obliterated. A new succession was in progress. Two zones were evident representing the two annual recessions since 1929. The youngest, nearest the water, was similar to Zones III and IV of 1926 with the addition of *Fuirena squarrosa* Michx. and *Drosera longifolia* L. The two-year-old zone was an associes of *Solidago*, *Coreopsis*, and *Eupatorium* and thus resembled Zone V of 1926. The pitch pines growing in Zone V in 1929, which are of especial interest in view of the discussion to follow, were thriving in 1931 about at the level of the maximum elevation of the lake in 1929.

CYCLIC VARIATION IN LAKE LEVEL

The common opinion of local residents that the level of the lakes in the region varies through a seven-year cycle had been largely verified by the observations made at Deer Pond. The campers there reported a maximum rise in 1922. The authors observed a low stage in 1926 followed by a rise with maximum in 1929, seven years after the 1922 maximum. During the years following this peak another recession was observed. In this connection the zones of *Pinus rigida*, dead and living, are of particular importance.

In 1926 two lines of dead pines were found at the lower limit of Zone VI. Since it is known that the water advanced to the upper limit of this zone in 1922 and since the trees appeared to have been dead for about four years, it is fairly safe to assume that they were killed in 1922. Counts of growth rings showed that there were two age groups among these pines. Those in the west transect had died at the age of 13 to 14 years while those in the east transect had lived for only 4 to 7 years. The fact that the oldest trees in each of these groups were 7 and 14 years old, respectively, strongly indicates that the one group lived on the precarious shore area during one cycle of lake-level fluctuation while those of the other lived during two cycles, both being killed by a subsequent maximum rise of unusual intensity in 1922. The following reconstruction has been made upon these assumptions.

An unusual maximum elevation of the lake occurred about the year 1908 which thoroughly denuded the shore to the upper limit of Zone VI (as defined in 1926). During the following recession pines became established on the exposed beach. The water soon returned and reached a maximum in 1915 but of lesser intensity than that of 1908. The pines were killed and subsequently obliterated up to the line of maximum rise which was at the level represented by the lower limit of Zone VI in 1926. Above this level the trees which were then of a maximum age of seven years con-

tinued to thrive through another seven-year cycle. During the lower water stages subsequent to this maximum of 1915 other pines seeded on the exposed beach in various places as in the east transect. When the water reached its unusual maximum in 1922 the pines in the west transect above the 1915 level were of a maximum age of 14 years while those in the east transect below that level were only seven.

One feature of these dead pines which cannot be satisfactorily explained is their occurrence in a definite line rather than in a wide zone as might be expected. No dead trees of lesser years were found lower down on the beach.

Fig. 7. Living *Pinus rigida* in 1931 slightly below the level at which dead pines occurred in 1926. They were established after the maximum elevation of the lake in 1922 which extended considerably above this; they mark the level of the maximum rise in 1929, and are from 6 to 8 years old. It is highly probable that these trees will be killed by the next elevation of the lake.

However, this does not detract from the reconstruction for it seems that the pines have a tendency to "line up" in a very narrow zone rather than populate a large area. This process was clearly seen in 1929 and 1931. Young pitch pines were found throughout Zone VI in 1926. However, in 1929 there was a definite line of these pines along the water's edge which represented the maximum of the cycle. The younger pines below probably had been washed away. At that time the pines at the water's edge were from 4 to 6 years old. The water did not rise sufficiently to kill these and they were found thriving in 1931 when they were photographed (fig. 7). On the basis of the above assumptions it may be predicted that these trees

will be killed by high water in 1936 when they will show about 14 annual growth rings.

Deep Pond is a kettle-hole lake the level of which is determined by the height of the water table. There seem to have been no records kept of the fluctuation in the level of the ground water in eastern Long Island. However, since this level is dependent upon the rainfall of the general locality, it is of interest to investigate the rainfall records. Figure 8 shows the annual rainfall for the years 1901 to 1930 at Setauket, about eight miles west of Deep Pond as obtained from the reports of the United States Weather Bureau. If there is a seven-year cycle in the fluctuation of the water table there should be a corresponding cycle in the variation in annual rainfall. Maxima and minima in these cycles will not be coincident as there will be a lag in the variation of the water table in response to variations in

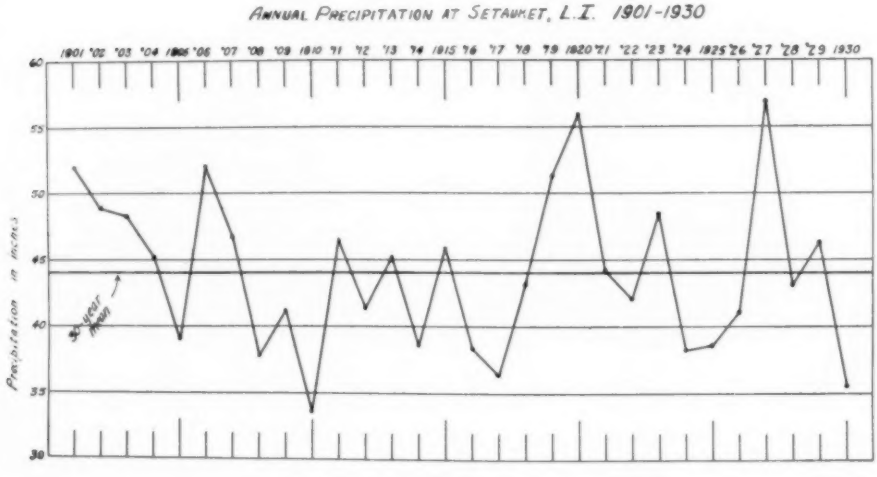

Fig. 8.

rainfall. The definite information available for the lake level for a limited number of years will permit a rough estimate of the amount of this lag.

The earliest authenticated maximum was in 1922. This probably was the result of the maximum rainfall in 1920 (fig. 8) representing a lag of about two years. The lowest level of the lake following this peak was in 1926. This was one and two years, respectively after the low annual precipitations of 1925 and 1924. The next maximum in lake level occurred in 1929. It was of comparatively low intensity and probably represents the effects of the high rainfall in 1927 showing a lag of about two years. Thus, in estimating the comparative level of the lake in past years from the

rainfall data it seems correct to consider a lag of about two years in the fluctuation of the height of the ground water.

In order to satisfactorily explain the occurrence of the pitch pines about the lake shore it was necessary to assume maximum water levels in 1908 and 1915, the one of 1908 of greater severity. It is now desirable to check these assumptions with the rainfall data. If there is a two year lag in the variation in lake level, high waters in those years must have been the result of maxima in the rainfall cycles in 1906 and 1913. Figure 8 shows that 1906 was a year of great rainfall and that 1913 was the last of a series of three fairly wet years. Thus it seems well founded that for the last twenty-five years at least, the rainfall of eastern Long Island has varied in cycles of about seven years causing a similar fluctuation in the water table and level of kettle-hole lakes which in turn has had a pronounced effect upon plant succession at the borders of the lakes.

SUMMARY

1. In 1926 a study of the vegetation bordering Deep Pond, a kettle-hole lake near Wading River, Long Island, showed that on the shore plant succession is periodically arrested and initiated by the fluctuations in the height of the ground water and thus in the level of the lake. Besides two vegetation zones in the water, four shore zones were observed representing four years of succession which occurred during a recession of the lake extending from 1922 to 1926.

2. In 1929 the arresting of one succession and the initiating of another caused by the rising lake level was observed. In 1931 a still later succession was found in progress as the lake once more receded.

3. From a study of zones of dead pitch pines bordering the lake it was deduced that maximum water levels had occurred in 1908 and 1915 and that, at least for the last twenty-five years, the level of the lake fluctuated through a cycle of about seven years. These conclusions were verified by a study of the rainfall records for Setauket, Long Island, which indicated a cyclic variation in rainfall corresponding to the cyclic fluctuation in the water table.

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INDEX TO AMERICAN BOTANICAL LITERATURE
1932–1933

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

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